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Psychomotor Performance in Man as Affected by High Oxygen Pressure (3 Atmospheres)

By

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Abstract

FRANKENHAEUSER, M., V. GRAFF-LONNEVIG and C. M. HESSER. *Psychomotor performance in man as affected by high oxygen pressure (3 atmospheres)*. Acta physiol. scand. 1960. *50*. 1—7. — Psychomotor performance (simple and choice reaction times and mirror drawing) of 10 subjects during exposure to oxygen at 3 atm was compared with performance under normal air breathing at 1 atm. No statistically significant differences in psychomotor performance under the two conditions could be demonstrated. Nor did performance show any tendency to deteriorate with time within the 30 min period employed. The possible importance of these observations as criteria of concomitant physiological events is discussed.

Several investigations have demonstrated that exposure to high oxygen pressure (HOP) can provoke convulsive seizures and unconsciousness. While individual susceptibility varies greatly, two generalizations appear to hold. First, the likelihood of eliciting seizures is increased the higher the pressure of oxygen, and second, the probability that seizures will occur is enhanced as the exposure to a given pressure is prolonged (see review of BEAN 1945). However, little is known as to the nature and site of the neurophysiological disturbances leading up to the convulsive state (STEIN 1955). According to GERSH (1945) the cerebral cortex as a whole is the site of origin of the motor seizure. If so, it seems reasonable to expect the convulsions to be preceded by a deterioration in performance.

The problem of a possible impairment of performance under HOP is thus important both from a theoretical, and, especially in the field of underwater physiology, from a practical point of view. So far, however, information as to performance changes under HOP has been derived from more or less casual observations (DAVIDSON 1926, DAMANT 1930, BEHNKE, JOHNSON, POPPEN and MOTLEY 1935, HALDANE and PRIESTLEY 1935, p. 356, HALDANE 1941, 1947) and quantitative, experimental data from human subjects seem not to be available.

The present investigation is an attempt to approach the physiological problems outlined above by the aid of psychological criteria. For this purpose performance in three psychomotor tasks as influenced by prolonged exposure (30 min) to oxygen at 3 atmospheres absolute (atm) was studied.

Methods

The experiments were carried out in a large recompression chamber with the subject seated in front of a table. Visual and auditory communications were established between the observers outside and inside the steel chamber. All the recordings were made by the observer outside the chamber. Commercial compressed air or oxygen were administered by means of a respiratory system comprising a mouthpiece, nose clip, a low resistance and low dead space breathing valve assembly (v. DÖBELN 1949), and two Douglas bags inside the chamber, each bag connected to a high pressure cylinder placed outside.

Subjects

Ten subjects (4 women and 6 men) aged 24 to 39 years (average 29 years) participated in the experiments. Four of the subjects were professional divers, the other six were laboratory technicians and medical students, two of whom were amateur divers.

Psychomotor tasks

Visual choice reaction time. Red, green, and yellow light signals were used as stimuli and presented automatically in the following manner. The three signals (neon signal bulbs, diameter 12 mm) were switched on by tuned frequency sensitive relays, one for each bulb. The bulbs were mounted on a gray metal square and arranged in the shape of a triangle, 5.5 cm from each other, the red bulb below the green, and the yellow bulb to the left of the two others. The signals were exposed at eye-level at a distance of 1 m from the subject. The relays actuating the bulbs were tuned to three different audio frequencies. Signals of these audio frequencies had been recorded on magnetic tape. When the tape was run through the recorder, the output initiated sequences of light stimuli by activating the relays.

The subject was instructed to respond to these stimuli by closing one of two Morse keys according to the following scheme:

Red light — left hand key
Green light — right hand key
Red and yellow light simultaneously — right hand key
Green and yellow light simultaneously — left hand key

There was thus a four-choice task, involving reversals of response in addition to the basic two-choice task.

The stimuli were presented in random order and were separated by an interval of irregular duration which averaged 2 secs. After one 10-sec period comprizing 5 stimuli, there was a 5-sec pause during which the sum of the 5 preceding reaction times was read to 0.001 sec on an electronic decade counter. This procedure was repeated 4 times during 1 min (20 responses). Each experimental session comprized three such 1-min periods, separated by about 11 min.

The following scores were obtained for each subject, both in the control and the O_2 experiments: a) Three *sub-scores*, each of which was the mean of the 20 reaction times recorded during one 1-min period, and b) one *total score*, which was the mean of all 60 reaction times obtained under the three 1-min periods.

Visual simple reaction time. The technique, frequency of stimulus presentation, and manner of score calculations were the same as in the choice reaction task except that one and the same stimulus (the yellow light) was invariably presented and the subject used one hand and one key only.

Mirror drawing[1]. The task was to move a stylus along a slit (visible only in a mirror) cut out in a metal plate so as to form a 5-pointed star. The slit had saw-tooth notches along both margins. The notches served to make the task more difficult by catching the stylus. The time to complete one run was measured by a stop-watch, and the time of contact between the stylus and the margins was recorded by the electronic decade counter. Four consecutive trials, separated by 45 secs, were performed, and, within one experimental session, the procedure was repeated 3 times at about 11 min intervals.

Time score was calculated as the mean of the time to complete the trials, and *error score* as the mean of the time of contact with the margins. *Sub-scores* for each subject were based on the 4 consecutive trials, *total scores* on all the 12 trials of one experimental session.

Experimental design

Each subject came to 3 sessions. The first session was devoted to indoctrination: preliminary training in psychological tasks at 1 atm was followed by trials at 3 atm with the subject breathing air through the respiratory system employed in the investigation.

The experiments proper were carried out during the two following sessions, one of which comprised trials during oxygen breathing at 3 atm (O_2 condition), the other one trials during air breathing at atmospheric pressure (control condition). Every other subject had the O_2 trials first.

The O_2 session was conducted according to the following scheme:

a) "Warming-up" trials during air breathing.
b) Pressure in chamber raised to 3 atm. Time for compression about 6 min.
c) 6 min allowed for temperature equilibrium.
d) Application of mouthpiece connected to the O_2 bag, followed by 5 min O_2 breathing.
e) Main experimental trials as follows:
   Choice reaction task 1 min
   Mirror drawing 4 »
   Simple reaction task 1 »

followed by two similar series. A 30-sec pause was inserted between successive tasks, and a 4-min pause between successive series. The two tasks on reaction time were performed in the reversed order by half of the subjects.

f) Decompression on air.

[1] The apparatus was designed by Dr. I. DUREMAN and Mr. E. ÖSTERBERG, the Psychological Laboratory, Psychiatric Clinic, Uppsala, Sweden, who kindly put the device at our disposal.

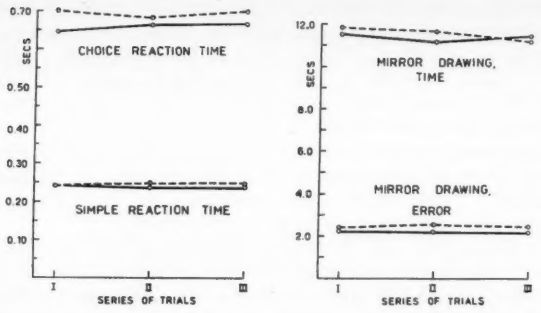

Fig. 1. Scores in psychomotor tasks at three consecutive series of trials during O_2 breathing at 3 atm (*points connected by hatched lines*) and during control conditions (air breathing at atmospheric pressure) (*points connected by continuous lines*). Consecutive trials *I*, *II*, and *III*, performed at intervals of about 11 min. Means of 10 subjects.

The same procedure and schedule were used in the control experiments, except that the subjects were breathing air at atmospheric pressure throughout the experimental period.

Results

The results obtained in three successive series of trials are depicted graphically in Fig. 1. No systematic changes in performance in the consecutive trials are seen in either condition. The lack of systematic changes during the control session indicates that the preceding practice had brought performance to a relatively stable level. Hence we may exclude the possibility that a deterioration in performance during the O_2 session was counterbalanced by practice effects, and may conclude that psychomotor performance did not vary with the time of exposure to HOP. A slight tendency for O_2 values to be higher (poorer performance) than control values is also seen.

Differences between O_2 and control data were examined further by calculating for each subject the average of his scores in the three consecutive series

Table I. Mean total scores (secs) in psychomotor tasks during O_2 breathing at 3 atm and during control conditions (air breathing at 1 atm). 10 subjects. Figures denote means ± S. E. of means

Task	O_2, 3 atm	Air, 1 atm
Simple reaction	0.245 ± 0.015	0.239 ± 0.011
Choice reaction	0.694 ± 0.031	0.657 ± 0.019
Mirror drawing, time	11.533 ± 0.874	11.371 ± 1.144
Mirror drawing, error	2.470 ± 0.437	2.201 ± 0.348

Table II. Intra-individual variability (secs) in psychomotor tasks during O_2 breathing at 3 atm and during control conditions

Task	O_2, 3 atm	Air, 1 atm
Simple reaction	0.115	0.095
Choice reaction	0.539	0.391
Mirror drawing, time	1.566	1.478
Mirror drawing, error	0.827	0.619

in the two conditions, respectively. The means of the total scores thus obtained are shown in Table I. Slight but consistent differences between the two sets of data are seen, the O_2 values being somewhat higher. To find out if the differences were statistically reliable, intra-pair mean differences between O_2 and control scores were submitted to the t-test.[1] None of the differences reached a statistically significant level; even the largest t-value, 1.384, as obtained in the choice reaction task, corresponds to $P \sim 0.20$ ($df = 9$).

Possible differences in intra-individual variability, which would be independent of the inter-individual variation, were examined by computing the square root of the average individual variance. As shown in Table II, the variability was somewhat greater in the O_2 than in the control experiments. However, t-tests based on intra-pair mean differences in individual variances, showed that the differences were not statistically reliable. The largest difference was in mirror drawing, error score, where $P \sim 0.10$ ($t = 1.841$, $df = 9$).

Discussion

The psychomotor tests employed in the present investigation did not reveal any clear-cut effects on performance of HOP (3 atm). This was true also when the time of exposure was extended to 30 min. The fact that no significant changes could be demonstrated does not, *a priori*, permit the conclusion that no effects occurred during the pre-convulsive period. However, the likelihood that our results are representative is increased, if it can be ascertained that the experimental conditions provided an adequate chance for the presumed effects to appear. Two main aspects of the experimental situation will be considered below: (a) The relative sensitivity of the psychomotor tests to disturbances in the central nervous system, and (b) the relative severity of the stress to which the subjects were exposed.

(a) That the three psychological tests used provide sensitive measures of impairment in performance has been established by earlier observations. Thus performance in the choice reaction task showed a statistically significant im-

[1] See *e. g.* FISHER (1948) for a description of the application of the t-test to differences in single individuals.

pairment in 7 subjects exposed to a moderate degree of gravitational stress (3 g) in a human centrifuge (FRANKENHAEUSER 1958). Likewise, performance in mirror drawing was significantly impaired, both in respect to time and error scores, in 9 subjects after intake of 200 mg pentobarbital as compared with performance after intake of 10 mg of metamphetamine (FRANKENHAEUSER 1959). Finally, SHILLING and WILLGRUBE (1937) found a slight but significant slowing of the simple reaction time at increased air pressures (5.5 atm or more).

(b) The next question is whether the oxygen pressure was high enough and/or the time of exposure sufficiently long to exert toxic effects. The results of previous investigators indicate that human subjects, during resting conditions inside a dry recompression chamber, may breathe pure oxygen at 3 atm with comparative safety for about 2 hours, whereas at 4 atm and more, the risk for convulsions rapidly increases (BORNSTEIN and STROINK 1912, THOMSON 1935, BEHNKE *et al.* 1935, BEHNKE, FORBES and MOTLEY 1936). However, in the present study one of the subjects got an attack with generalized convulsions and unconsciousness after 17 min of oxygen breathing at 3 atm. This incident is of considerable interest, partly because such rapid development of convulsive attacks seems to be rare under the conditions employed, and partly because records are available of the subject's performance up to the moment of onset of the attack. (The data obtained from this subject, since incomplete, were omitted from the results described in the previous section.) Inspection of his performance data prior to the onset of the attack reveals no sign of deterioration when compared with results from previous control trials. Even data on mirror drawing obtained after the subject had already — according to his own retrospective account — experienced some twitching around the mouth, did not differ noticeably from those obtained in the corresponding trial under control conditions. Though, naturally, much importance cannot be attached to data from one single experiment, it is nevertheless interesting to note that psychomotor performance can be maintained at a high level up to the critical point, when overt symptoms of oxygen poisoning are noticeable.

From the fore-going it may be inferred that the experimental conditions of the present study were well adapted for tracing even slight effects on psychomotor performance of HOP. Hence, it should be permissible to conclude that, in subjects at rest, no significant impairment in psychomotor performance develops during the pre-convulsive period of exposure to HOP (3 atm). It may be inferred, then, that during this period no major disturbances occur in those areas of the cerebral cortex which control psychomotor performance. Support for this view is provided by the observations of LAMBERTSEN *et al.* (1953 a, b) that in man, after O_2 breathing for 15—30 min at 3—3.5 atm, the average O_2 tension of cerebral venous blood did not exceed 75 mm Hg. Under these conditions the larger mass of brain cells may therefore not be exposed to toxic tensions of oxygen, although the arterial O_2 tension amounts to 1,700—2,100 mm Hg. Data have also been presented (SONNENSCHEIN, STEIN and PEROT 1953)

indicating that, in cats exposed to increasing O_2 pressure, the cortical O_2 tension rises at a much slower rate, to show a sharp and extreme rise just prior to or during the development of seizures.

We wish to express our appreciation to Miss BERIT JONASSON and Mr. T. HOLM for their technical assistance.

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Different Pools of Catecholamines Stored in the Adrenal Medulla

By

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Abstract

HILLARP, N.-Å. *Different pools of catecholamines stored in the adrenal medulla.* Acta physiol. scand. 1960. *50.* 8—22. — The nucleotide content of the adrenal medulla (cow, horse, pig and cat) and its changes *post mortem* were analyzed by means of ion exchange and paper chromatography. The experiments showed that the catecholamines may exist in excess of the adenosinephosphates, which indicates that a not inconsiderable fraction of the amines cannot be stored together with an equivalent amount of adenosinephosphates in the way found in pure amine granules of high density. Examinations of the intracellular distribution of nucleotides and amines indicated that at least some of these amines do not exist "free" in the cytoplasm but are bound in an unknown way to granules of lower density and apparently of lower stability. The probable existence of different pools of stored amines is discussed.

The catecholamines in the adrenal medulla of all birds and mammals that have as yet been examined have been found to be stored together with an equivalent amount of adenosinephosphates, mainly ATP (*cf.* HILLARP and THIEME 1959, HILLARP, JÖNSSON and THIEME 1959). This result was obtained by analysis of amine granules isolated by means of density gradient centrifugation. These granules probably represent those with maximum storage of amines. Calculations from data obtained in the first studies of unfractionated medulla (HILLARP, HÖGBERG and NILSON 1955, FALCK, HILLARP and HÖGBERG 1956) however, show, that the amines may be present in excess of the adenosinephosphates. Until last year, however, this nucleotide deficit was thought

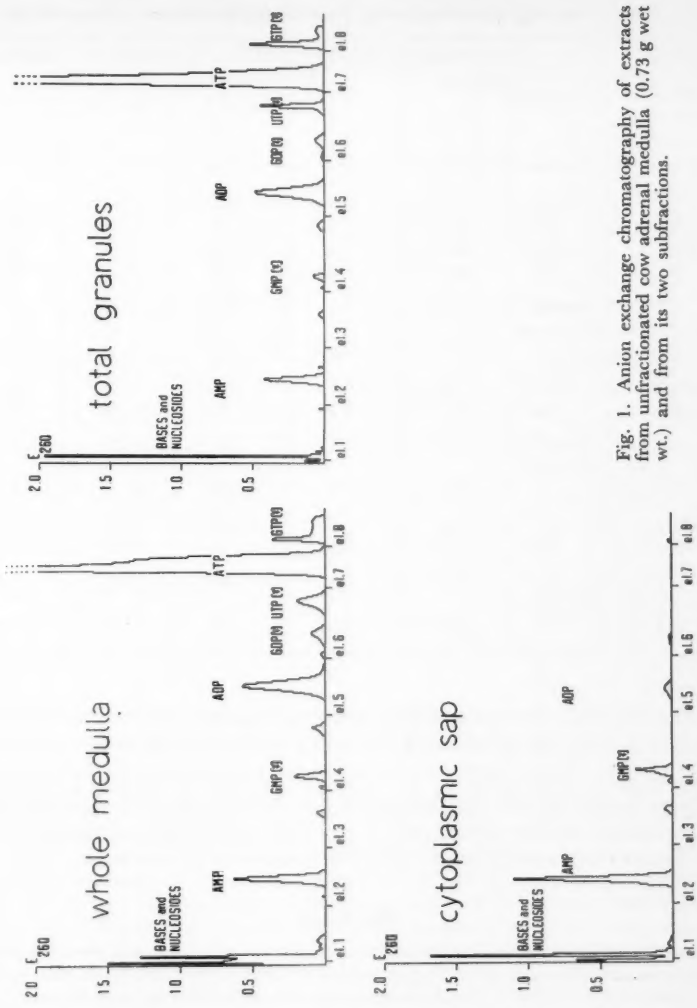

Fig. 1. Anion exchange chromatography of extracts from unfractionated cow adrenal medulla (0.73 g wet wt.) and from its two subfractions.

to be due either to nucleotide losses during the analytical procedures or to a postmortal ATP breakdown. Studies in other laboratories (BLASCHKO *et al.* 1956, SCHÜMANN 1957, 1958) showed an ATP deficit of varying magnitude in several instances. In these cases, however, a biological assay was used and, besides, this did not admit of determinations of ADP and AMP or other breakdown products of ATP.

Table I. Content (μmoles) of catecholamines, nucleotides, hypoxanthine and inorganic and acid-

Experiment		Catechol-amines	ATP	ADP	AMP	IMP
Normal medulla	1	71.5	9.90	1.92	1.11	0.0
	2	81.5	10.9	1.93	1.23	0.0
	3	68.7	8.60	1.57	1.04	0.0
	4	80.5	9.80	1.65	1.16	0.0
Mean	Per g medulla	*75.5*	*9.8*	*1.77*	*1.13*	*0.0*
	Per 100 μmoles amines	*100*	*13.0*	*2.35*	*1.50*	*0.0*
Medulla kept at +37° 2 and 3 h	5	78.8	10.5	2.02	1.41	0.0
	6	78.2	9.4	2.40	1.34	0.0
Mean	Per g medulla	*78.5*	*10.0*	*2.21*	*1.37*	*0.0*
	Per 100 μmoles amines	*100*	*12.7*	*2.82*	*1.75*	*0.0*
Crushed medulla + 37° 2 h	7	78.2	1.70	0.87	0.99	1.0
	8	78.5	4.10	1.03	0.78	0.38
Mean	Per g medulla	*78.3*	*2.90*	*0.95*	*0.88*	*0.69*
	Per 100 μmoles amines	*100*	*3.70*	*1.21*	*1.11*	*0.88*

It was recently thought possible, however, that the observed deficit might be a real one existing in the living cell owing to the presence in the cytoplasmic sap of a pool of "free" amines which might be of importance for the regulation of the synthesis, storage and release of the catecholamines. Closer and more careful analysis of the content and intracellular distribution of catecholamines and nucleotides in the adrenal medulla was therefore necessary.

Methods

Extraction procedure. After shooting of the animals the adrenals were removed without delay (cow, and horse: within 30, pig: within 15, sheep: within 10 and cat: within 2 min) and chilled with ice. The medullas (pooled from several animals in the case of cow, horse and pig) were thoroughly crushed and extracted several times with perchloric acid (PCA; final concentration 0.4 N). The extract was immediately neutralized to about pH 6 with potassium carbonate and left overnight at —4° C. The precipitated perchlorate was spun down and washed with water.

Cell fractionation. In each experiment about 10 g of cow medulla was homogenized with a loose-fitting plastic pestle for about 15 sec in 30 ml of 0.3 M sucrose. To remove unbroken tissue and cells but at the same time to prevent losses of amine granules the homogenate was centrifuged at $800 \times g$ for only 3 min. The supernatant was centrifuged at $38{,}000 \times g$ for 30 min. The supernatant ("cytoplasmic sap", including much

labile phosphorus in cow adrenal medulla before and after incubation at +37°

Hypoxanthine	Ad.-Phosphates μeq Per cent of amines	Ad.-Phosphates +IMP+Hypoxanthine	Inorganic P	Acid-labile P	
				Observed	Calculated Per cent of observed
2.9	67	15.8	19.3	25.6	99
2.7	64	16.8	20.4	29.6	96
2.5	60	13.7	18.7	24.3	96
2.6	58	15.2	19.2	27.8	94
2.7	*62*	*15.4*	*19.4*	*26.8*	*96*
3.6	*62*	*20.4*	*25.8*	*35.5*	*96*
3.6	65	17.5	23.2	26.8	97
4.0	61	17.1	26.4	27.1	93
3.8	*63*	*17.3*	*24.8*	*26.9*	*95*
4.8	*63*	*22.1*	*31.6*	*34.3*	*95*
12.0		16.6	47.0	9.70	63
10.0		16.3	42.4	14.0	66
11.0		*16.4*	*44.7*	*11.8*	*64*
14.1		*21.0*	*57.0*	*15.1*	*64*

microsomal material) and the sediment ("total large granules", including *i. a.* the amine granules, mitochondria and some nuclei) were extracted with PCA as described above.

Determination of nucleotides, nucleosides and bases. An aliquot of the neutralized extract containing about 50 μmoles of catecholamines was passed through a cation exchange column (Dowex 50, 150 to 300 mesh, 30 × 8 mm, pH 6). Besides catecholamines, only small amounts of substances absorbing at 250, 260 or 280 mμ were retained. The effluent and washings were brought to about pH 9 with ammonium hydroxide and passed through an anion exchange column (Dowex 2, 200 to 400 mesh, 200 × 6 mm, formate form). The elution (5 ml fractions, flow-rate about 10 ml/hr) was performed with formic acid, containing increasing concentrations of sodium formate (BERGKVIST and DEUTSCH 1954, BERGKVIST 1957) and was followed by absorption measurements at 250, 260 and 280 mμ. A molecular extinction coefficient of 14,500 was used for the adenosinephosphates.

The nucleotides were tentatively identified with aid of their elution positions and ultraviolet absorption spectra in acid and alkali, as compared with pure nucleotides. Furthermore the adenosinephosphates were identified by high voltage paper electrophoresis, paper chromatography and by cation exchange chromatography of the bases after hydrolysis (see below).

The ultraviolet absorbing fractions eluted with 0.02 M formic acid (eluting agent 1 in Fig. 1) were evaporated *in vacuo*, dissolved in N HCl and re-chromatographed on a Dowex 50 column (see below). The main compound (52 to 61 per cent of the E_{260} of

Table II. Content (μmoles/g wet wt.) of catecholamines and inorganic and acid-labile phosphorus in cow adrenal medulla before and after incubation at +37°

		Catechol-amines	Inorganic P	Acid-labile P	Acid-labile P Per 100 μmoles amines
Medulla before incubation	A	76.6	19.5	26.4	34.5
	B	95.6	21.1	32.9	34.4
	C	84.0	15.0	29.4	35.0
	D	77.5	20.2	25.1	32.4
	Mean	*83.4*	*18.9*	*28.4*	*34.1*
Medulla after incubation + 37° 2 h	A	82.2	21.8	29.5	35.9
	B	93.3	22.1	32.6	34.9
	C	85.5	20.6	28.2	33.0
	D	76.5	25.1	25.1	32.8
	Mean	*84.4*	*22.4*	*28.8*	*34.2*

the combined fractions from extracts of normal cow glands) was identified as hypoxanthine by its ultraviolet absorption in acid and alkali and by paper chromatography (see below).

An aliquot of the original extract was hydrolyzed in N HCl (+ 100°, 1 hour) after removal of the catechol amines by a small Dowex 50 column at pH 6. The hydrolysate was chromatographed on a cation exchange column (Dowex 50, 150 to 300 mesh, 200 × 6 mm, H^+) using 1, 2 and 4 N HCl as eluting agents (*cf.* COHN 1951, WALL 1953). Hypoxanthine, guanine and adenine were recovered in expected amounts and eventually identified by paper chromatography.

Paper chromatography of the bases was performed with systems a to d and that of the nucleotides with systems d to g: a) *iso*propanol — HCl (WYATT 1951), b) *iso*propanol — NH_3 (HERSHEY, DIXON and CHASE 1953), c) *n*-butanol — acetic acid, d) *iso*propanol — ammonium sulphate solution, e) *n*-propanol — NH_3, f) *iso*butyric acid — NH_3, g) ethanol — ammonium acetate buffer pH 3.8 (c to g according to BERGKVIST 1956).

The values obtained for the adenosinephosphates on high voltage paper electrophoresis (see HILLARP, JÖNSSON and THIEME 1959) of the original extract were about 15 per cent higher than those observed on anion exchange chromatography. This is due to an incomplete separation of the nucleotides.

Precautions taken to detect and prevent losses of nucleotides. When not otherwise stated all procedures used for obtaining a neutralized extract were done at 0° and without any delay. The neutralized extract was kept at —30° for at most 3 days before analysis. The procedures, including the cation exchange step for removal of the catechol amines, were tested (re-extractions of tissues and perchlorate precipitates and determinations of ultraviolet absorption, inorganic and acid-labile phosphorus before and after each step) and found to give inconsiderable losses. Anion exchange chromatography of pure adenosinephosphates showed good recoveries (96 to 102 %) and no appreciable breakdown of ATP. In three extracts (1:M, 1:G and 2:M in Table III) the sum of E_{260}

of the different compounds eluted from the column was 98, 102 and 95 per cent respectively of the E_{260} of the amine free extract. Finally, the values for acid-labile phosphorus calculated from the obtained amounts of nucleotides agree well with those directly determined in the original extracts (1 to 6 in Table I).

Other analyses. Duplicate determinations of inorganic (P_0) and acid-labile (P_8) phosphorus (LEPAGE 1951) and of catecholamines (EULER and HAMBERG 1949) were performed. The catecholamines retained by the cation exchanger were eluted with N HCl and determined by ultraviolet absorption as well.

Reference substances. Pure nucleotides from the Pabst Laboratories and pure l-adrenaline and l-noradrenaline generously supplied by Rhone-Poulenc were used. The standard solutions were checked by determination of ultraviolet absorption.

Results

Nucleotide and catecholamine content of unfractionated cow medulla

Like other cells the medullary cell contains several nucleotides other than adenosinephosphates. Although they have not been definitely identified it is however clear from the data obtained on anion exchange chromatography (Fig. 1) and from determinations and calculations of P_8, that of the nucleotides the adenosinephosphates are clearly predominant quantitatively.

To secure reliable data four different extracts of pooled medullas were carefully analyzed (Table I:1—4) with precautions to prevent nucleotide losses. Since the adenosinephosphates — at least ATP — are mostly used in the storage of an equivalent amount of amines, their content is also expressed in μmoles/100 μmoles of amines. These values have more significance and show less variability than those calculated per weight of medulla.

The analyses showed that the amounts of amines present largely excceds the adenosinephosphates, the equivalent content of which is on an average only 62 per cent of the amine content. Thus, no more than about 60 per cent of the amines can have been present in a storage complex built of *i. a.* equivalent amounts of amines and adenosinephosphates.

There are two possible sources of error in the analyses:

a) Losses of nucleotides due to analytical procedures. The tests performed showed (see Methods) that this cannot be a major factor.

b) Losses due to postmortal conversion or breakdown of the adenosinephosphates in the medulla. This is a serious source of error, the significance of which is difficult to estimate without experiments (next section).

Postmortal changes of the nucleotide content in the cow adrenal medulla

The medulla shows a high ATPase and ADP transphosphorylase activity which rapidly break down the stored ATP if it is released from the amine granules (HILLARP 1958). To test the possibility that ATP is broken down in this or other ways before cooling and extraction of the glands, three experiments were performed.

Table III. Content (μmoles/g wet wt.) of catecholamines, nucleotides, hypoxanthine and Exp. 1 and 2: normal medulla. Exp. 3: medulla kept at +37° 3 h.

Exp.	Fraction	Catechol-amines	ATP	ADP
1	"Total large granules" (G)	64.0	10.4	1.63
	"Cytoplasmic sap" (S)	17.5	0.0	0.0
	G+S	81.5	10.4	1.63
	Unfractionated medulla (M).....	81.5	10.9	1.93
2	G	58.7	7.60	1.05
	(G washed 2 ×) G_w	46.2	6.70	0.70
	S	21.8	0.0	0.16
	G+S	80.5	7.60	1.21
	M.....	80.5	9.80	1.65
3	G	50.0	9.00	1.47
	S	28.2	0.0	0.0
	G+S	78.2	9.00	1.47
	M.....	78.2	9.40	2.40

From 4 suitable glands a piece of pure medulla was dissected both before and after incubation of the glands for 2 hours at +37° and P_0 and P_8 were determined. The results (Table II) show that there was an increase of P_0 but no corresponding decrease of P_8. Thus, the stored ATP seems to be remarkably stable in glands kept at body temperature.

Since transphosphorylations might be possible, the pooled medullas from several glands kept at +37° for 2 to 3 hours were analyzed. No obvious decrease or breakdown of the adenosinephosphates was found (Table I: 5—6).

At most only small amounts of nucleosides and bases (such as inosine, adenine and adenosine) besides hypoxanthine were present in normal glands. The hypoxanthine content was fairly constant (2.7 μmoles/g medulla). In the medullas kept at +37° for 2 to 3 hours it increased only about 1 μmole (Table I: 5—6) which shows that no appreciable quantities of the adenosinephosphates stored together with amines were broken down to hypoxanthine. If — on the other hand — the medullas were thoroughly crushed to release the granule-bound nucleotides, the largest part of ATP was broken down during incubation at +37° for 2 hours. It is seen (Table I: 7—8), however, that a corresponding increase of hypoxanthine occurred and also some inosinemonophosphate (IMP) appeared. No appreciable quantities of other possible breakdown products (*e. g.* adenine, adenosine, inosine) were found. Thus the

inorganic and acid-labile phosphorus in unfractionated cow adrenal medulla and its two subfractions

AMP	Hypoxanthine	Ad.-phosphates μeq Per cent of amines	Ad.-phosphates + hypoxanthine	Inorganic P	Acid-labile P	
					Observed	Calculated Per cent of observed
0.76	0.90	75	13.7	4.15	26.6	98
1.85	1.9	21	3.8	19.5	0.0	
2.61	2.8		17.5	23.6	26.6	
1.23	2.7	64	16.8	20.4	29.6	96
0.45	0.40	59	9.5	4.40	21.2	93
0.35	0.30	64	8.0	2.74	17.3	100
2.76	2.5	27	5.4	23.6	0.0	
3.20	2.9		14.9	28.0	21.2	
1.16	2.6	58	15.2	19.2	27.8	94
0.77	1.3	84	12.5	4.35	24.1	94
1.85	2.8	13	4.7	26.0	0.0	
2.62	4.1		17.2	30.4	24.1	
1.34	4.0	61	17.1	26.4	27.1	93

medulla has enzymes of high activity not only for dephosphorylation of the adenosinephosphates but also for their deamination. Calculations based on the values found for normal glands clearly show that the entire amounts of the adenosinephosphates that disappeared were recovered as hypoxanthine and IMP and that the breakdown did not proceed any further. This also appears from the fact that the sum of adenosinephosphates, hypoxanthine and IMP was found to have the same value in the incubated glands as in the normal ones (Table I).

These three experiments thus show that a) the ATP stored in the amine granules does not break down to any appreciable extent when glands are kept at body temperature even for hours; and that b) the ATP that may disappear postmortally is in all probability recovered as hypoxanthine (or as ADP, AMP and IMP). Since normal glands contain hypoxanthine, a breakdown of granule-bound ATP may have occurred postmortally. The real ATP content should then be about 17 μmoles/100 μmoles of amines. However, even if the entire content of hypoxanthine, ADP and AMP is included as ATP in the calculations (Table I: 1—4), it is evident that about 20 per cent of the amines in the living medulla cannot have been stored together with an equivalent amount of adenosinephosphates.

Intracellular distribution of catecholamines and nucleotides in the cow adrenal medulla

During the homogenization some of the amine granules are damaged (HILLARP, LAGERSTEDT and NILSON 1953). As seen from the data in Table III the ATP and ADP released in this way were recovered as AMP in the fraction "cytoplasmic sap". This fraction also contained (Fig. 1) the largest part of hypoxanthine but only small amounts of nucleotides other than AMP and guanosinemonophosphate (?) (GMP). Practically the whole nucleotide content of the medulla was thus recovered in the fraction "total large granules" (Fig. 1). The guanosinetriphosphate (?) (GTP) and uridinetriphosphate (?) (UTP) found here are probably located in the mitochondria and nuclei which in other tissues are known to contain these nucleotides.

In the two experiments with normal medulla 79 and 73 per cent of the amines in the low-speed supernatant was recovered in the granule fraction. In the third experiment in which the glands were kept at +37° for 3 h before fractionation, the corresponding figure was 64 per cent. This decrease of granule-bound amines is due to some unknown processes which occur during the incubation (HILLARP, LAGERSTEDT and NILSON 1953). Previous studies have shown that the amine granules obtained by means of density gradient centrifugation contain amines and adenosinephosphates in equivalent amounts. The analyses of the total granules in this work (Table III) however, show, that not all the granule-bound amines can be stored in this way. The adenosinephosphate deficit is so large that it cannot be explained on the basis of nucleotide losses during the experimental procedures. This is further proved by the recoveries of adenosinephosphates in the two subfractions of the medulla.

Thus there seem to exist two different fractions of granule-bound amines. The results obtained in the experiment with incubated glands support this view. Here it is seen that the adenosinephosphate deficit is smaller than in the two other experiments. This is most reasonably explained on the basis that the amines released from the granules due to the incubation originated from a fraction of amines stored without adenosine phosphates. This is in agreement with the result obtained in the previous section, namely that ATP is remarkably stable during such incubations. Consequently, an ATP breakdown cannot be the cause of the amine release. This view is further supported by the finding (exp. 2 in Table III) that washings of the granules remove more amines than ATP. These findings also indicate that this amine fraction is less stable than the fraction in which amines and adenosinephosphates are stored together. It is also clear, however, that the amines in the former fraction are not so loosely bound that they are easily removed by washing of the granules. This was confirmed in another experiment where the total granule fraction was left at 0° C for 24 h, then washed twice and analyzed. Here the adenosinephosphate deficit was at least 10 per cent (3 exp. not shown in the tables on account of incomplete determinations).

Table IV. Content (μmoles) of catecholamines, nucleotides and inorganic and acid-labile phosphorus in adrenal medulla

Pig and sheep: mean of two determinations. Cat 1: mean of four determinations (8 animals). Cat 2: mean of two determinations (10 animals).

		Catecholamines	ATP	ADP	AMP	Hypoxanthine	Ad.-phosphates μeq Per cent of amines	Inorganic P	Acid-labile P
Horse	Per g medulla	70.5	7.03	2.42	1.30	2.9	54	20.0	22.6
	Per 100 μmoles amines	100	10.0	3.45	1.85	4.1	54	28.4	32.1
Pig	Per g medulla	87.5	13.1	3.20	4.40	1.25	81	19.2	38.7
	Per 100 μmoles amines	100	15.0	3.66	5.03	1.45	81	21.9	44.2
Sheep ("total large granules")	Per g medulla	51.0	10.0	1.33	0.50	—	88		
	Per 100 μmoles amines	100	19.6	2.61	0.98	—	88		
Cat 1	Per 100 μmoles amines	100	13.2	6.6	4.1	—	81	36.1	33.1
Cat 2	Per g medulla	48.0						18.6	16.1
	Per 100 μmoles amines	100						38.8	33.6

Nucleotide and amine content of the adrenal medulla in other animals

The *horse* medulla (Table IV) showed practically the same nucleotide pattern as the cow medulla, the main difference possibly being a lower ATP content in the former. The adenosinephosphate deficit was also found to be larger (46 per cent).

The possibility that an appreciable ATP breakdown might occur postmortally could not be confirmed in the experiments with cow medulla. Nevertheless it was thought of importance to examine glands that could be removed from the animal body, cooled and extracted much more rapidly than was the case in these experiments. This was possible by use of adrenals from pig, sheep and cat. The pertinent data are found in Table IV.

Pooled medullas from *pig* glands (obtained about 15 min after the shooting of the animals) were examined. They had a considerably lower hypoxanthine and a higher adenosinephosphate — especially AMP — content than those found in cow glands. Even in the pig medulla, however, there is a clear adenosinephosphate deficit amounting to about 20 per cent.

From two male *sheep* (killed by a blow on the head) the adrenals could be removed about 10 min after death. The nucleotide content of the total granule

fraction was determined by use of a short anion exchange column. The values obtained are therefore too high owing to contamination of the adenosinephosphates with other nucleotides. Since this contamination was found to have fairly constant values (15 to 20 per cent of the three adenosinephosphate fractions) in the analyses on cow, horse and pig, the values found in sheep were reduced by 15 per cent. A small adenosinephosphate deficit (12 per cent) was found.

The most interesting data were obtained on examination of *cat* glands. In two experiments 6 and 4 animals respectively were killed by shooting and the adrenals were immediately cooled and dissected at 0°. In spite of this the P_s content was found to be low, in fact as low as in the cow medulla. In another experiment four different extracts of pooled medullas from two animals in each were examined for their maximum content of adenosinephosphates by use of a short exchange column. The values obtained were reduced by 15 per cent (see above) but must still be too high since the P_s-values calculated from ATP + ADP are the same as the observed ones. This should mean that no other acid-labile phosphates than ATP and ADP could have been present which is quite improbable. It is thus evident that there is an adenosinephosphate deficit, amounting to at least 20 per cent, in the cat medulla.

The *rat* adrenal medulla has been examined previously (HILLARP, JÖNSSON and THIEME 1959) but the values found for the adenosinephosphates are probably too high. (It was then not known that the presence of other nucleotides yields values which might be as much as 15 per cent too high.) Here also there is probably a deficit, although a small one.

Discussion

Since amine granules isolated by density gradient centrifugation from adrenals of both birds and mammals were found to contain catecholamines and adenosinephosphates in equivalent amounts, it was thought that the entire content of granule-bound amines was stored in the same way. The analyses in this study of unfractionated medulla from various animals unexpectedly showed, however, that the amines are present in excess of the adenosinephosphates. The nucleotide deficit may be great (> 20 per cent) and — as shown — cannot be due to losses in the analytical procedures.

This deficit could, however, have arisen postmortally owing to an ATP breakdown. The high stability of the granule-bound ATP does not support this view, but it cannot be excluded that a less stable fraction might have broken down. But as shown above, this should in all probability have given rise to an accumulation of equivalent amount of hypoxanthine (and/or ADP, AMP and IMP). Thus if the whole content of these compounds is added to ATP, one should get a value for the ATP content that could maximally have been present in the living medulla. In the cow medulla there would never-

theless be a deficit amounting to about 20 per cent (Table I). Still more convincing evidence for the existence of a deficit was obtained in the experiments with cat medulla. Here a large adenosinephosphate deficit was found in spite of the fact that the animals were killed by shooting and the adrenal glands could be cooled and dissected within a few minutes after death. It is further of importance that the deficit could be proved to exist even by calculations based on the content of acid-labile P.

There is thus strong evidence supporting the view that the entire content of amines in the living medullary cell cannot be stored together with an equivalent amount of adenosinephosphates. The only possible objection to this would be that there might occur a very rapid ATP breakdown within the first few minutes after the death of the animals. It is hard to see, however, how this could be achieved without leaving any traceable products, especially since the anoxic medulla (in the cow at least) does not seem able to break down adenosinephosphates any further than to hypoxanthine.

The presence of catecholamines in excess of adenosinephosphates might be thought to imply that part of the amines exist "free" in the cell cytoplasm instead of being bound in granules. In fact the starting-point of this work was the hope that it might be possible by careful examinations of the content and intracellular distribution of nucleotides and amines to settle the important problem (*cf.* HILLARP 1960) whether there exists a pool of "free" amines in the medullary cell. The unexpected result of the examinations of the total granule fraction which — besides other particles — contains all the amine granules was, however, that there is a deficit also in the pool of granule-bound amines. This deficit cannot reasonably be due to a postmortal ATP breakdown. The structure of the amine-ATP storage complex (*cf.* HILLARP 1959) is inreconcilable with the view that ATP might have disappeared from the granules without a corresponding amine release. The conclusion seems inescapable that part of the amines stored in granules has a storage mechanism not involving ATP. These amines may represent a not inconsiderable part, since some of the adenosinephosphates located in the total granule fraction clearly must belong to other particles, *e. g.* mitochondria.

At present it is not possible even to speculate on the mechanism binding the amines in this new type of granules. It seems probable that the amines also in these granules are stored together with an equivalent amount of acid. The phosphoric acid content is much too low for this (Table III) and nucleotides other than adenosinephosphates also seem to exist in too small amounts — especially since part of them at least must be assumed to be located in the mitochondria. The experiments suggest that amines are more readily released from this granule fraction than from the ATP-amine storage structure, but they also show that the amines are kept by a mechanism preventing them from being easily removed through washing of the granules. The two granule types also differ in another respect. The "new" granules must have a lower

density than the pure granules isolated by density gradient centrifugation, since these have equivalent amounts of amines and adenosinephosphates (HILLARP and THIEME 1959). This explains why this "new" fraction has been unnoticed in the previous studies.

It is difficult to decide whether the two granule-bound amine fractions are two entirely different pools or whether they represent two stages in the storage process, the formation of the ATP-amine complex being the final step. The latter hypothesis is at least not supported by the finding that a parallel storage of amines and ATP occurs after a depletion (unpublished experiments). The role of the presumably less stable amine fraction is also difficult to decide. Its main significance at present is therefore that is shows the probable existence of different amine pools. This must be taken into account in studies of synthesis, storage and release of catecholamines.

When testing the biological activity of intravenously injected intact amine granules BLASCHKO, HAGEN and WELCH (1955) observed that only 15 to 25 per cent of the amines were readily released. BLASCHKO *et al.* (1956) made another puzzling observation, *viz.* that the top layer obtained by centrifugation of the large granule fraction has an ATP content which is often considerably lower than the bottom granules. These findings now get a reasonable explanation from the presence of two different fractions of granule-bound amines.

The origin of the catecholamines recovered in the cytoplasmic sap is a difficult but important problem. They constitute a fraction which is at least partly composed of amines released from the granules by postmortal processes occurring in the medulla and by a damage of the granules during the isolation — in particular probably the homogenization — procedure (HILLARP, LAGERSTEDT and NILSON 1953). In fact these experiments provided evidence for the view that the entire content of amines might be granule-bound in the living cell. On the other hand, they could not exclude the existence of a small pool of "free" amines. A damage of the granules, *e. g.* disruption during the homogenization step, must give a release of not only amines but also of an equivalent amount of ATP, which ought to be recovered as AMP in the fraction "cytoplasmic sap". Experiments 1 and 3 in Table III show, however, that such a damage needs not occur to any larger extent. Unfortunately it cannot be decided from the studies of the amine and nucleotide distribution to what extent the "free" amines originate from a postmortal release of bound amines since a release might take place from the pool of amines bound in granules without ATP. The experiments only show that the amine release which occurs progressively in glands kept at $+37°$ is not accompanied by any appreciable release or breakdown of ATP and that the amines are thus probably liberated from the apparently less stable pool of amines stored without ATP.

It does not seem unreasonable, however, that part of the amines recovered in the supernatant represents a third pool of amines which may exist "free"

in the cytoplasmic sap. The findings concerning the sites of amine synthesis in the cells support this view. It has been shown that both the decarboxylation (BLASCHKO *et al.* 1955) and the final methylating step (KIRSCHNER and GOODALL 1957, MASUOKA, CLARK and SCHOTT 1958) probably occur in the cytoplasmic sap of the medullary cell. It is thus possible that new-formed amines are to a certain extent accumulated in the cytoplasm before being stored in the amine granules or secreted. Another argument is the finding that, during secretion, stored amines must be released from the granules to the cytoplasmic sap before being finally transported out of the cell (HILLARP, HÖKFELT and NILSON 1954). Thus both new-synthesized and released amines may contribute to a small pool of free amines. In all experiments in this laboratory with different animals whose adrenals can be rapidly removed, homogenized and fractionated, at least 5 to 10 per cent of the amines have been recovered in the "free" fraction in spite of all precautions to prevent secondary changes in the intracellular distribution of amines. These and other experiments which will be reported in another paper (BERTLER, HILLARP and ROSENGREN 1960) at least indicate the existence of such a pool.

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Inhibitory Effects of Hydrochloric Acid in Antrum and Duodenum on Gastric Secretory Responses to Insulin Hypoglycemia in Pavlov Pouch Dogs

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Abstract

ANDERSSON, S. *Inhibitory effects of hydrochloric acid in antrum and duodenum on gastric secretory responses to insulin hypoglycemia in Pavlov pouch dogs.* Acta physiol. scand. 1960. *50.* 23—31. — In Pavlov pouch dogs with the antrum and duodenum excluded from the normal gastrointestinal passage, gastric secretion was stimulated by insulin hypoglycemia. On instillation of hydrochloric acid into either the antrum or the duodenum the secretory responses were markedly inhibited. The inhibitory mechanisms are discussed.

In earlier investigations (ANDERSSON 1960 a, b), acid pH in the duodenum inhibited fasting and postprandial gastric secretion from Pavlov and Heidenhain pouches. The inhibitory influence was independent of the vagal innervation to the gastric pouch. Acid milieu in the antrum, on the other hand, caused slight or no inhibition. However, the mechanism behind the fasting secretion was not known, and the secretory responses following a test meal were caused by both vagal and intestinal secretory stimuli. It was not possible, therefore, to determine the mechanism of the inhibition elicited from the antro-duodenal areas studied.

Since insulin hypoglycemia is considered to induce gastric secretion via the activation of vagal impulses, it was thought to constitute a more definable type of secretory stimulus. In the present experiments the secretion was accordingly stimulated by the intravenous injection of insulin, and the effects thereon of acid infusions into the antrum and duodenum were studied.

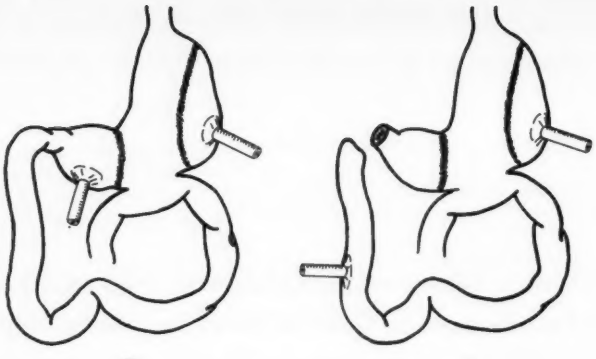

Fig. 1. Operative preparation of the dogs used:

I. (Dog 50) Pavlov pouch; mucosal wall between the corpus and antrum; gastrojejunostomy and antral cannula.

II. (Dogs 75, 126 and 137) Pavlov pouch; mucosal wall between the corpus and antrum; antral fistula and cannula in the duodenum.

Methods

Four adult dogs weighing between 15 and 20 kg were used. At an initial operation all four were provided with Pavlov pouches. Subsequently a double wall of mucosa was constructed along the antrum-corpus border and the gastrointestinal passage was restored by gastrojejunostomy. At a third operation a cannula was inserted into the antrum of one of the dogs (no. 50; Fig. 1: I); the other three (nos. 75, 126 and 137) were prepared as follows: The pylorus was transected and the duodenal end closed. The antral opening was brought out to the abdominal surface as a cutaneous fistula. A cannula was inserted into the duodenum about 10 cm distal to its upper end (Fig. 1: II). For a more detailed description of operative and experimental procedures, the reader is referred to earlier papers (Andersson *et al.* 1958; Andersson 1960 b).

Insulin was administered intravenously; the dose used for the different dogs was so adjusted as to produce a submaximal secretory response and was in the range of 0.1—0.4 I.U. per kg body weight. Throughout the secretory response the blood sugar level was determined every half hour by the anthrone method.

In dog no. 50, N/10 HCl was used for infusions into the antrum-duodenum (about 150 ml per hour). Acid of the same strength was also used for perfusion of the antrum of dogs 75, 126 and 137. At a perfusion rate of approximately 100 ml per hour the intra-antral pH varied between 1.2 and 1.5. For reasons mentioned in a previous paper (Andersson 1960 b), N/5 HCl was used for duodenal instillations in these three animals. Instillation of approximately 100 ml acid per hour reduced the intra-duodenal pH to about 2. For a description of the perfusion and instillation technique, the reader is referred to Andersson 1960 a and b. The infusion of acid into the antrum or duodenum began concurrently with the administration of insulin and proceeded for between 1 and 2 hours.

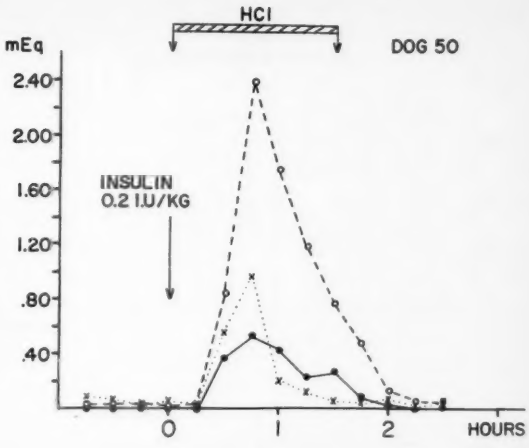

Fig. 2. Inhibition of secretory response to insulin hypoglycemia in a Pavlov pouch dog by instillation of hydrochloric acid into the antrum-duodenum. Secretory response before (●——●, 3 exp.) and after (○——○, 4 exp.) exclusion of antrum-duodenum, and with concomitant instillation of N/10 HCl into excluded antrum-duodenum (×···×, 3 exp.).

Table I. Hourly secretory responses to insulin hypoglycemia in a Pavlov pouch dog before and after antrum-duodenum exclusion and after exclusion with concomitant instillation of hydrochloric acid into the excluded areas

Dog 50. Dose of insulin: 0.2 I.U./kg

		Secretion (mEq total acid)			
		Control period (one hour)	Response to insulin		
			1st hr	2nd hr	
	Exp. no.			1/2 hr	1/2 hr
Before antrum-duodenum exclusion	1	0	0.80	0.18	0
	2	0	1.63	0.35	0 13
	3	0	1.61	0.97	0.22
	Mean	0	1.35	0.50	0.12
After antrum-duodenum exclusion	1	0	5.06	2.32	0.86
	2	0	4.62	1.60	0.68
	3	0	4.77	2.50	0.53
	4	0.31	5.55	1.44	0.34
	Mean	0.08	5.00	1.97	0.60
After antrum-duodenum exclusion with concomitant instillation of N/10 HCl into those areas during the 1st and half of the 2nd hour	1	0.35	**1.96**	**0.19**	0.03
	2	0	**1.04**	**0.13**	0.04
	3	0.22	**2.34**	**0.18**	0.13
	Mean	0.19	**1.78**	**0.17**	0.07
In percent of mean secretory response after antrum-duodenum exclusion		—	**36**	**9**	12

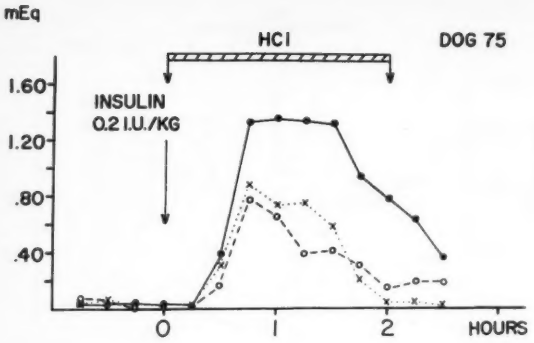

Fig. 3. Inhibition of secretory response to insulin hypoglycemia in a Pavlov pouch dog by instillation of hydrochloric acid into the antrum and duodenum.
●——●: Control (5 exp.);
○ - - -○: N/5 HCl in duodenum (4 exp.);
× . . . . ×: N/10 HCl in antrum (4 exp.).

Results

A. *Instillation of Acid into the Antrum-Duodenum*

A marked increase of insulin-stimulated secretion from Pavlov pouches occurs, as shown by Uvnäs *et al.* (1956) and Andersson *et al.* (1958), if the antrum and duodenum are excluded from the normal gastrointestinal passage. Instillation of HCl into the excluded sector inhibits this hypersecretion.

Fig. 2 shows the mean secretion following insulin in dog 50 before and after exclusion of the antrum and duodenum (plus antral cannulization). When N/10 HCl was infused into the antrum-duodenum at a rate of approximately 150 ml per hour the secretory response was reduced to about the same level as that observed before exclusion of the antrum and duodenum. Table I shows the hourly secretion in each experiment under the different secretory conditions.

Owing to technical difficulties no reliable determinations of the antro-duodenal pH were practicable during instillations of acid in this dog.

B. *Instillation of Acid into the Duodenum or Antrum*

In order to ascertain if the inhibition was elicited by contact of the acid with the antral or the duodenal mucosa or with both, dogs 75, 126 and 137 were prepared, with surgical separation of the antrum and duodenum (see Fig. 1: II).

a) Acid in the Duodenum

The effect of infusion of acid into the duodenum was studied in 12 exp. Reduction of the intra-duodenal pH invariably caused a pronounced inhibition of the secretion. Fig. 3 shows the mean secretory response of a Pavlov pouch to insulin with and without concomitant intra-duodenal instillation of acid. Instillation of about 100 ml N/5 HCl per hour (intra-duodenal pH approximately 2) inhibited the secretory response by an average of 62 per cent.

Table II. Hourly secretory responses to insulin hypoglycemia in Pavlov pouch dogs with and without concomitant instillation of hydrochloric acid into the antrum and duodenum

A. Dog 75. Dose of insulin: 0.2 I.U./kg

		Secretion (mEq total acid)		
		Control period (one hour)	Response to insulin	
	Exp. no.		1st hr	2nd hr
Controls	1	0.15	3.96	3.22
	2	0.05	2 44	4.79
	3	0.05	3.38	5.14
	4	0.14	2.73	4.48
	5	0.05	2.91	4.19
	Mean	0.09	3.08	4 36
N/5 HCl in duodenum during the 1st and 2nd hours	1	0.40	**1.83**	**0.32**
	2	0.01	**1.96**	**1.88**
	3	0.01	**0.91**	**1.81**
	4	0.07	**1.62**	**0.93**
	Mean	0.12	**1.58**	**1.24**
In percent of mean for controls		—	**51**	**28**
N/10 HCl in antrum during the 1st and 2nd hours	1	0.14	**1.35**	**1.42**
	2	0.08	**2.06**	**1.49**
	3	0.22	**1.52**	**1.65**
	4	0.01	**2.71**	**1.72**
	Mean	0.11	**1.91**	**1.57**
In percent of mean for controls		—	**62**	**36**

b) Acid in the Antrum

The secretion was also inhibited appreciably on acidification of the antrum. 11 exp. were conducted with perfusion of acid into the antrum. Fig. 3 shows that perfusion of the antral pouch by approximately 100 ml N/10 HCl per hour (intra-antral pH 1.2—1.5) caused a marked inhibition of the secretion, averaging 58 per cent.

For a more detailed study of the secretory responses to insulin hypoglycemia in each dog see Table II, which shows the hourly gastric output of total acid in the different experiments.

Instillation of physiological saline into the antrum or duodenum had no inhibitory effect upon the secretion.

There was no difference in principle between the results if the secretion was expressed in milliequivalents of free HCl instead of milliequivalents of total acid.

Table II (Cont.)

B. Dog 126. Dose of insulin: 0.1 I.U./kg

		Secretion (mEq total acid)		
		Control period (one hour)	Response to insulin	
	Exp. no.		1st hr	2nd hr
Controls	1	0.03	1.55	1.45
	2	0	1.64	1.10
	3	0.36	1.95	1.41
	Mean	0.13	1.71	1.32
N/5 HCl in duodenum during	1	0.03	**0.60**	0.59
the 1st hour	2	0	**0.51**	0.51
	3	0.16	**0 23**	0.33
	4	0.10	**0.02**	0.01
	Mean	0.07	**0.34**	0.36
In percent of mean for controls		—	**20**	27
N/10 HCl in antrum during	1	0.03	**0.59**	0.93
the 1st hour	2	0.03	**0.77**	0.92
	3	0	**0.25**	0.83
	4	0	**0.43**	1.10
	Mean	0.02	**0.51**	0.95
In percent of mean for controls		—	**30**	72

C. *Effect of Pylorotomy on Insulin-Induced Secretory Response*

In the intervals between the different operations on dogs 75 and 126 the secretory behavior of the Pavlov pouches in response to a dose of insulin was repeatedly determined. It is evident from Table III that exclusion of the antrum and duodenum gave rise to an expected increase of the secretion which averaged 111 and 427 per cent respectively (*Cf.* UVNÄS *et al.* 1956). Pylorotomy did not appreciably influence these hypersecretory responses; the change averaged + 9 and — 23 per cent respectively (see Table III).

Discussion

The aforegoing experiments show that the heightened secretory responses to insulin hypoglycemia in Pavlov pouch dogs with the antrum and duodenum surgically excluded from the normal gastrointestinal passage, are effectively inhibited when hydrochloric acid is instilled into either of those areas.

Table II (Cont.)
C. Dog 137. Dose of insulin: 0.4 I.U./kg

		Secretion (mEq total acid)		
		Control period (one hour)	Response to insulin	
	Exp. no.		1st hr	2nd hr
Controls	1	0	0.25	0.44
	2	0.01	0.63	0.74
	3	0	0.30	0.75
	Mean	0	0.39	0.64
N/5 in duodenum during	1	0.20	**0.11**	**0.19**
the 1st and 2nd hours	2	0	**0**	**0**
	3	0	**0**	**0**
	4	0	**0**	**0**
	Mean	0.05	**0.03**	**0.05**
In percent of mean for controls		—	**8**	**8**
N/10 HCl in antrum during	1	0	**0.05**	**0.03**
the 1st and 2nd hours	2	0	**0.01**	**0.33**
	3	0	**0**	**0**
	Mean	0	**0.02**	**0.12**
In percent of mean for controls		—	**5**	**19**

Inhibition Induced from the Duodenum

Intra-duodenal infusion of acid has been reported (PINCUS *et al.* 1944, CODE and WATKINSON 1955) to cause a significant inhibition of insulin-stimulated secretion. It was recently shown (ANDERSSON 1960 a, b) that fasting as well as postprandial secretions were effectively inhibited in both Pavlov and Heidenhain pouch dogs on instillation of acid in the duodenum. It was not surprising, therefore, to find that the insulin-stimulated secretion also could be inhibited. Since the same duodenal mechanism may well have caused the inhibition of the different types of secretion, the reader is referred to earlier papers (ANDERSSON 1960 a, b) for a more detailed discussion of the character of that mechanism.

Inhibition Induced from the Antrum

Marked inhibition of the secretory responses was observed also when the antrum alone was perfused with acid. The same observation had been made by SHIMIZU, MORRISON and HARRISON (1958), who were able to inhibit insulin-stimulated secretory responses from Pavlov pouches by perfusing an isolated antral pouch with acid. Earlier WOODWARD *et al.* (1954) had discounted the idea that insulin-induced secretion from a total stomach pouch could be inhibited by acidifying the antrum. The conclusions of WOODWARD *et al.* are, however, very much open to question, for those authors conducted only one

Table III. Mean secretory responses to insulin hypoglycemia in Pavlov pouch dogs before (A) and after (B) exclusion of antrum and duodenum and after pylorotomy (C)

Dog no.	Insulin	A		B			C		
		Number of experiments	Mean secretory response	Number of experiments	Mean secretory response	Change	Number of experiments	Mean secretory response	Change compared with B
	I.U./kg		mEq		mEq	%		mEq	%
75	0.2	2	3.67	3	7.76	+ 111	5	8.43	+ 9
126	0.2	3	1.03	3	5.43	+ 427	2	4.16	− 23

experiment on each of three animals and, moreover, did not report any control experiments; thus they had no possibility of recording anything but total inhibition. The present experiments, like those of SHIMIZU *et al.*, show that insulin-stimulated secretion is only partially inhibited by acidification of the antrum.

It was remarked in the introduction that in a previous investigation (ANDERSSON 1960 b) the writer had not been able to influence appreciably the postprandial secretory responses from Pavlov pouches by intra-antral perfusion of acid. It is interesting, therefore, to note that the effect of antral acidification on insulin-stimulated secretion conflicts with those observations, particularly in that the same dogs were used in both runs of experiments. Secretion induced by insulin hypoglycemia is presumably of a type conditioned solely by the vagus, whereas a secretory response to a test meal must be attributed to both vagal and intestinal secretory stimuli. The varying degrees of susceptibility to antral inhibition which different types of secretion exhibits, thus lend support to the assumption that the antral inhibitory mechanism may interfere with vagal secretory stimuli. Whether this inhibition is due to reduced release of gastrin from the antral mucous membrane, or to the activation of some other antral inhibitory mechanism, cannot be decided.

The investigation demonstrates, too, that division of the pylorus to create an isolated antral pouch did not appreciably affect the hypersecretory tendency of the Pavlov pouch which arose after exclusion of the antrum and duodenum. This observation rules out the possibility that the increased secretory responses to insulin following the exclusion were due solely to stimulation of the antral mucosa by regurgitated duodenal contents, an alternative which UVNÄS *et al.*, 1956 could not entirely dismiss.

The depression of insulin-induced secretion which, as shown here, results from acidification of the antrum as well as duodenum, corroborates the aforementioned authors' view that the increased secretion following exclusion of the antrum and duodenum is attributable to exclusion of both antral and duodenal inhibitory mechanisms.

Conclusions

Insulin-stimulated gastric secretion from Pavlov pouches was effectively inhibited by the instillation of hydrochloric acid into the duodenum. This finding further corroborates the evidence for an intra-duodenal, pH-sensitive inhibitory mechanism. The secretory responses were considerably reduced on perfusion of the antrum with hydrochloric acid. This finding conflicts with earlier investigations by the writer, in which fasting secretion and postprandial secretion were not inhibited appreciably by acidifying the antrum. The varying degrees of responsiveness to antral inhibition that were shown by different types of secretion are considered to support the assumption that the antral inhibitory mechanism interferes only with the vagal phase of gastric secretion.

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Electrophysiological Studies on Gamma Motoneurones

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Abstract

Eccles, J. C., R. M. Eccles, A. Iggo and A. Lundberg. *Electrophysiological studies on gamma motoneurones.* Acta physiol. scand. 1960. *50*. 32—40. — Gamma motoneurones supplying the muscle spindles of limb muscles of cats have been investigated by microelectrodes either extracellular or intracellular, and shown to lie interspersed with the alpha motoneurones of that muscle. The spike potential of the gamma motoneurones resembles that of alpha motoneurones both in having an IS—SD composition and in being followed by a long after-hyperpolarization. When activated either by repetitive antidromic impulses, or by a prolonged depolarizing current, the gamma motoneurones give some spike potentials at much higher frequencies than the alpha. It was confirmed that the gamma motoneurones differ from the alpha in receiving no monosynaptic excitatory action, but there are polysynaptic excitatory and inhibitory actions from other afferents.

The motoneurones supplying small efferent fibres to limb muscles which have been identified as the gamma efferent fibres to muscle spindles (Eccles and Sherrington 1930, Leksell 1945, Kuffler, Hunt and Quilliam 1951) have hitherto been studied by recording impulses in their axons. Such investigations of gamma motoneurones have provided a wealth of information about the excitatory and inhibitory actions exerted on them by muscle and cutaneous afferent impulses (Hunt 1951, Kobayashi, Oshima and Tasaki 1952, Eldred and Hagbarth 1954, Hunt and Paintal 1958), and of the descending controls of their activity (Granit and Kaada 1952, Eldred, Granit and

MERTON 1953). With the exception of a brief report (ECCLES 1955) there has been no description of their investigation by microelectrodes. The account given here is assembled from incidental observations that were made during other investigations over several years.

Method

The experimental procedures were similar to those described for investigations with microelectrodes on the alpha motoneurones of cat limbs (COOMBS, ECCLES and FATT 1955, COOMBS, CURTIS and ECCLES 1957, ECCLES, ECCLES and LUNDBERG 1957a). The ventral roots were usually intact so that gamma motoneurones could be identified by antidromic invasion on stimulation of muscle nerves (cf. ECCLES *et al.* 1957b).

Results

Identification of gamma motoneurones

In the standard procedure antidromic volleys in efferent fibres were generated by stimulation of muscle nerves at strengths maximal for the gamma as well as for the alpha efferent fibres. Owing to their slower conduction velocity the impulses in gamma efferent fibres entered the spinal cord through the ventral roots 2 or more milliseconds later than the slowest alpha impulses; hence it was expected that the antidromic spike potentials of alpha and gamma motoneurones would be clearly distinguishable. As a microelectrode was slowly advanced through the ventral horn, it often recorded extracellularly one or more spike potentials with a latency which identified the cells as gamma motoneurones. For example in Fig. 1A there is a complex of spike potentials with a latency of 5.0 to 8.8 msec, while the antidromic spike potential for the alpha motoneurones of this same muscle had a latency of about 2 msec. Suitable variation of the strength of stimulus applied to the muscle nerve revealed that the late complex of spikes was produced by five all-or-nothing units which had latencies of 5.0, 5.8, 6.1, 6.3 and 8.8 msec, corresponding to conduction velocities of 35, 30.5, 29, 28 and 20 m/sec, which are all in the usual range for gamma efferent fibres. The threshold stimulus strengths relative to the alpha threshold for these five gamma fibres corresponded approximately to the values expected, being in the range of 5.6 to 12 times the threshold for the most excitable fibres of the plantaris nerve, the group I a fibres (cf. LEKSELL 1945). Usually only one or two gamma motoneuronal responses could be detected with any position of the microelectrode. A grouping of as many gamma motoneurones in close proximity as in Fig. 1 was very unusual. The conduction velocities for impulses in the axons of the gamma motoneurones of our series have ranged from 20 to 40 m/sec, which is in good agreement with the ranges given by LEKSELL (1945), HUNT (1951), KUFFLER *et al.* (1951) and HUNT and PAINTAL (1958). It also agrees with the velocities to be expected for fibres with diameters ranging from 2.5 to 8μ (ECCLES and SHERRINGTON 1930, REXED and THERMAN 1948).

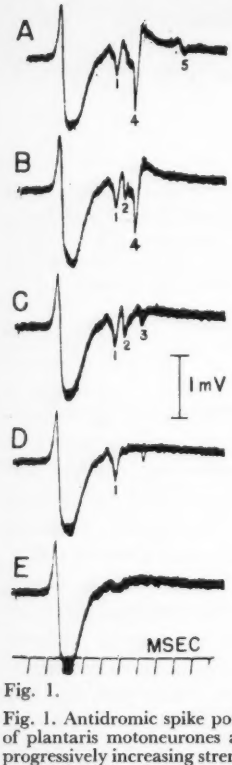

Fig. 1.

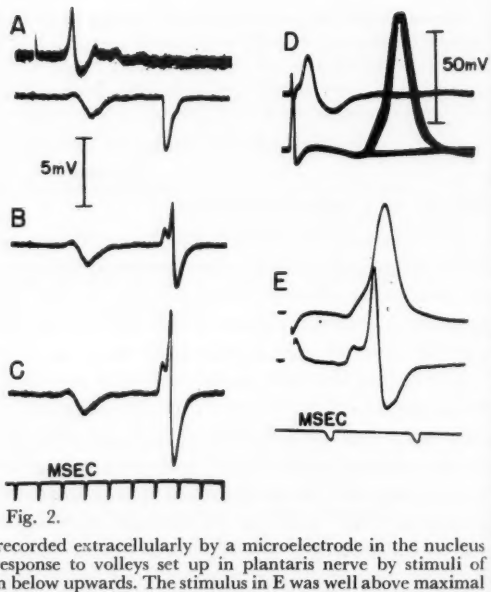

Fig. 2.

Fig. 1. Antidromic spike potentials recorded extracellularly by a microelectrode in the nucleus of plantaris motoneurones and in response to volleys set up in plantaris nerve by stimuli of progressively increasing strength from below upwards. The stimulus in E was well above maximal for the alpha motor fibres which evoked the typical large diphasic field potential, downward deflection signalling negativity. With further increase in the stimulus from D to A, the various later all-or-nothing spike potentials labelled 1 to 5 appeared, corresponding to five gamma motoneurones. Each record was formed by superposition of about forty faint traces. The large 4 tended to submerge units 2 and 3 in A and B.

Fig. 2. Lower trace of A shows extracellular record of a gamma motoneurone belonging to medial gastrocnemius (there being also the initial diphasic spike due to alpha motoneurones as in Fig. 1) and in the upper trace the antidromic spike potential recorded by an electrode on the surface of the spinal cord. The microelectrode was then gradually advanced, giving the partly intracellular records of B and C with an initial positively directed spike having IS and SD components. D and E show large spike potentials recorded intracellularly and evoked by antidromic invasion of a gamma motoneurone which was identified by the high threshold of the motor axon in the ventral root (about 3.3 times the alpha axon threshold) and its slow conduction velocity (about 30 m/sec). Membrane potential was —60 mV. Note double composition of spike; this is especially clear in the electrically differentiated record (E, lowest trace). Upper trace in D is the surface potential as in A.

Location of gamma motoneurones

When the spike potentials of the gamma motoneurones were recorded outside the cells, the extracellular field potentials of the alpha motoneurones of the same muscle were large (Fig. 1, 2A—C). The fields of all other moto-

neuronal nuclei were always smaller (compare the extracellular spikes marked by arrows in Fig. 4B, D and F). It can therefore be concluded that the gamma motoneurones of a muscle are located within the nucleus of alpha motoneurones supplying that muscle.

Sprague (1951) found in Rhesus monkeys that, 9 to 12 days after section of the spinal nerves, small chromatolysed neurones were present in the ventro-medial zone of the ventral horn. He suggested that these were gamma motoneurones. Both SPRAGUE (1951 and BALTHASAR (1952) have also reported that there are numerous small motoneurones interspersed among the large motoneurones in the ventral horn. Recent work makes it probable that the small chromatolysed neurones of Sprague were Renshaw cells, which have now been located in the ventro-medial zone by both physiological and anatomical work (ECCLES, FATT and KOKETSU 1954, SZENTAGOTHAI 1958). The gamma motoneurones interspersed with the large motoneurones may correspond to the small cells of SPRAGUE (1951) and BALTHASAR (1952) but may have failed to develop chromatolysis. Alternatively the population of chromatolysed cells reported by SPRAGUE (1951) may have included both alpha and gamma motoneurones. Evidently there is a need for further histological work.

Intracellular recording from gamma motoneurones

As shown in Fig. 2, 3 and 4 it was occasionally possible to insert a microelectrode into a gamma motoneurone. The resting membrane potential was greater than —50 mV in only a few gamma motoneurones; the low values were attributed to the larger injurious effect of the microelectrode on such relatively small neurones. However, in a few gamma motoneurones fairly extensive intracellular investigations were possible.

The spike potentials generated by antidromic activation have been up to 76 mV in height and have invariably been compounded of a small initial and later large spike (Fig. 2D., E; 3A), corresponding precisely to the IS—SD spikes of alpha motoneurones (ARAKI and OTANI 1955, FATT 1957, FUORTES, FRANK and BECKER 1957, COOMBS *et al.* 1957). It may therefore be assumed that in gamma motoneurones too there is a membrane with a low threshold over the initial segment and a much higher threshold over the soma and dendrites. As with depolarized alpha motoneurones (COOMBS *et al.* 1955), the spike potential usually declined onto a brief after-hyperpolarization (Fig. 2D, 4A), which may likewise be attributed to the phase of high potassium conductance during and just after the falling phase of the spike.

A much longer after-hyperpolarization followed this brief phase in the two motoneurones whose electrical responses were recorded on a sufficiently slow time base. For example in Fig. 3G, H, the after-hyperpolarization is seen to be about 70 msec in duration and to have a time course comparable with that of fast alpha motoneurones (ECCLES, ECCLES and LUNDBERG, 1958). HUNT and PAINTAL (1958) investigated the reflex excitability of six gamma moto-

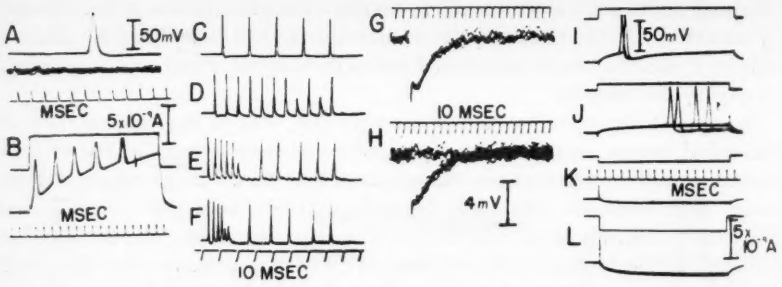

Fig. 3. Intracellular records of a gamma motoneurone of plantaris muscle. A shows antidromically evoked spike potential, while in B the large depolarizing current pulse of upper trace (4.5×10^{-9}A) caused a repetitive firing at almost 400/sec. C to F show responses to repetitive antidromic impulses at 66, 153, 315 and 520/sec respectively. G and H are superimposed traces at high amplification and slow sweep speed in order to show the afterhyperpolarization following the spike potential, the stimulus in H being just at threshold for the gamma axon. I to L are records showing potentials produced by rectangular current pulses, depolarizing in I and J and hyperpolarizing in K and L, as shown by the records lying above the potential trace. Note spike potentials in I and J. Time scales are shown for the various records, and the same potential scale applies to all records except G and H. The scale for the current pulses is also shown.

neurones after invasion by an antidromic impulse, and described two types of recovery. With four cells the recovery was complete in 10 msec, while with two others it was as slow as would be expected with an after-hyperpolarization of about 70 msec (Fig. 3, G, H). Furthermore, a fairly long depression after a spike potential was indicated by the observation that a background spontaneous discharge was depressed for 20 msec or longer after an intercurrent reflex discharge (HUNT and PAINTAL 1958).

Repetitive activation of gamma motoneurones

Gamma motoneurones have in general exhibited higher maximum frequencies of discharge than alpha motoneurones (HUNT 1951, KOBAYASHI *et al.* 1952, HUNT and PAINTAL 1958). The last authors reported peak frequencies of 300—500/sec for the brief reflex responses to cutaneous or muscle afferent volleys, and peak frequencies of 100 to 150/sec in response to natural stimuli. It was therefore to be expected that the gamma motoneurones would follow higher frequencies of antidromic activation, than do the alpha motoneurones. At all frequencies up to 520/sec Fig. 3 F, the full IS—SD spike potentials were generated by the first few impulses, and thereafter there was an initial phase of IS—SD blockage of frequencies above 150/sec (Fig. 3 D). Finally, most of the higher frequency antidromic impulses produced only the small M spikes, which made a ripple on the baseline (Fig. 3 E); nevertheless the full IS—SD spike still occurred at a frequency of almost 100/sec. Alpha motoneurones invariably are much poorer at responding with IS—SD spike potentials when

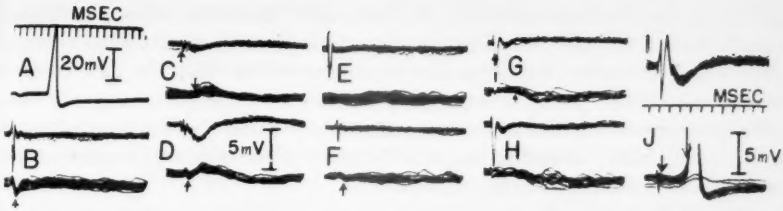

Fig. 4. A—H are intracellular records from a gamma motoneurone of flexor digitorum longus. A shows spike potential and B—H are superimposed traces in response to volleys from various muscle nerves at higher amplification, but with same time scale as in A. B, flexor digitorum longus volley with stimulus just below threshold of the gamma axon. C, D, extensor digitorum brevis volleys, in D maximal for alpha motor fibres, so giving the initial antidromic field potential that is marked by arrow. E, F, G maximal for group I afferent fibres for nerves to biceps-semitendinosus, extensor digitorum longus and quadriceps muscles, the quadriceps stimulus being still stronger in H. J shows intracellular response evoked in another gamma motoneurone by a dorsal root volley that gave the surface potential shown in I. The latency of the spike potential is measured between the two arrows. Note separate time scales for I, J, and potential scale for J. Same potential scale for B—H.

activated antidromically at high frequencies (Brock, Combs and Eccles 1953), the maximum frequency of response being at the most 30—40/sec during sustained activation. This difference may indicate that with the gamma motoneurones the after-hyperpolarization does not build up to such a depressant level as it does with the alpha during repetitive activation. A further factor would be the absence of recurrent inhibitory action on gamma motoneurones (Granit, Pascoe and Steg 1957, Hunt and Paintal 1958), which was also observed with several of the gamma motoneurones of the present series (cf. Fig. 3H).

In the present series there was one doubtful instance of a gamma motoneurone receiving recurrent inhibition. This cell was a soleus motoneurone that had an axon conduction velocity of 35 m/sec and a threshold that would identify it as a gamma motor axon; for it certainly has been rare to find soleus alpha motoneurones with conduction velocities below 50 m/sec. Yet this motoneurone had a large prolonged after-hyperpolarization (240 msec in duration), resembling in this respect soleus alpha motoneurones. However, it received a total recurrent inhibitory effect that was extraordinarily small for a soleus alpha motoneurone. The total as formed by arithmetical addition of the IPSP's from the individual muscle nerves being 4 mV, as against totals of 18 and 21 mV for the two typical soleus alpha motoneurones in the same animal (R. M. Eccles, Iggo and Ito, unpublished).

Only one gamma motoneurone was investigated by passing rectangular current pulses through the intracellular microelectrode. As shown in Fig. 3 I—L, the responses have closely resembled those obtained with alpha motoneurones. There was approximate symmetry of potentials produced by de-

polarizing and by hyperpolarizing currents, until the former caused sufficient depolarization to generate spike potentials. A very intense depolarizing current gave a high frequency discharge (almost 400/sec in Fig. 3B). The time courses of the membrane potential changes produced by hyperpolarizing and depolarizing currents indicate that the membrane had an electric time constant of about 2.3 msec, or rather longer if the effect of the dendrites is allowed for (cf. COOMBS, CURTIS and ECCLES 1959).

Synaptic action on gamma motoneurones

In most experiments with intracellular recording from gamma motoneurones the dorsal roots were cut and were not mounted for stimulation. However, it has been confirmed that gamma motoneurones differ radically from the alpha motoneurones in that they are not monosynaptically excited by group Ia impulses (ELDRED *et al.* 1953, HUNT and PAINTAL 1958). Nor did we observe any effect which with certainty could be ascribed to Ib afferents. The records in Fig. 4 A—H show that, on a gamma motoneurone of flexor digitorum longus there was after a brief central latency an excitatory action from the nerve to extensor digitorum brevis (central latency 2.2 msec as measured between the arrows in Fig. 4C). There is however a cutaneous component in this nerve which may have caused this excitatory effect. The only other significant effect was an inhibitory action (latency 3.3 msec) by relatively low threshold quadriceps afferent fibres (G, H). There was no significant synaptic action from the group I and II afferent fibres of its own muscle (B) or of its other antagonist (F). A relatively short latency discharge (2.3 msec) in response to a dorsal root volley is shown by another gamma motoneurone (Fig. 4J), which is seen to arise from an excitatory synaptic potential. The small size of EPSP required to excite can be attributed to the low membrane potential of this motoneurone, but may be common also in normal cells since many of them are firing spontaneously (HUNT 1951, KOBAYASHI *et al.* 1952, HUNT and PAINTAL 1958, VOORHOEVE 1960).

Discussion

The experimental observations have shown that gamma motoneurones and the alpha motoneurones for a particular muscle are interspersed in the ventral horn and that the gamma motoneurones resemble alpha in their general properties. They have, however, a much poorer tolerance of intracellular electrodes and a higher maximum frequency of discharge. There is also an important difference with respect to the synaptic connections they receive: gamma motoneurones fail to receive any of the group Ia monosynaptic connections (cf. ELDRED *et al.* 1953, HUNT and PAINTAL 1958), which are invariably received by alpha motoneurones from the appropriate muscle nerves (ECCLES *et al.* 1957b, R. M. ECCLES and LUNDBERG 1958). Since the

gamma and alpha motoneurones supplying a muscle are closely interspersed in the same motoneurone nucleus, it can be asked: What factors during development result in the alpha motoneurones receiving a rich supply of monosynaptic connections and the gamma motoneurones receive none? There is also an absence of recurrent inhibitory synapses from Renshaw cells, though some alpha motoneurones also lack recurrent inhibition (ECCLES *et al.* 1954, R. M. ECCLES, IGGO and ITO, unpublished). The alpha motoneurones with smallest axons, however, received the largest recurrent inhibition (R. M. ECCLES *et al.* 1960), whereas the gamma notoneurones, which have still smaller axons, received none, with the doubtful exception of the soleus motoneurone referred to above. In respect of other synaptic contacts, alpha and gamma motoneurones of a particular muscle are not dissimilar. For example, both are similarly acted on during flexor and crossed extensor reflexes (HUNT 1951, KOBAYASHI *et al.* 1952, ELDRED and HAGBARTH 1954, HUNT and PAINTAL 1958). Evidently some highly specific process operates in preventing the establishment both of monosynaptic excitatory synapses and of Renshaw inhibitory synapses on gamma motoneurones.

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Acta physiol. scand. 1960. 50. 41—48

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In Vitro Release of Histamine from Cat Skin by Compound 48/80

By

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Abstract

WESTERHOLM, B. *In vitro release of histamine from cat skin by compound 48/80.* Acta physiol. scand. 1960. *50.* 41—48. — A method for studying histamine releasing activity is described. Chopped cat skin was incubated with liberator and the histamine released was assayed on guinea-pig ileum. The mechanism of action of compound 48/80 on this preparation was studied. It was shown that histamine release by 48/80 was dependent on time, concentration, temperature and pH. There were temperature and pH optima, though they were not well defined with the doses used. The release could be blocked by certain enzyme inhibitors as the amino group reagent, ninhydrin, and the sulfhydryl group blocking substance, allicin. The observations are considered to support the hypothesis that 48/80 acts by means of an enzyme mechanism.

Compound 48/80 has been shown to release histamine from various animal tissues. The most sensitive methods described are perfusion of cat skin flap (FELDBERG and MONGAR 1951) and perfusion of cat paw (HÖGBERG *et al.* 1956). These are not well adapted for quantitative studies of histamine releasing activity, however, since no controls are available and since usually there is marked tachyphylaxis.

In the present paper a method for studying the effect of compound 48/80 on chopped cat skin is reported. Cat skin was used because it has a high content of releasable histamine (FELDBERG 1956).

The skin preparation made possible quantitative comparisons in that numerous samples were obtainable. It was also possible to study the effect of pH,

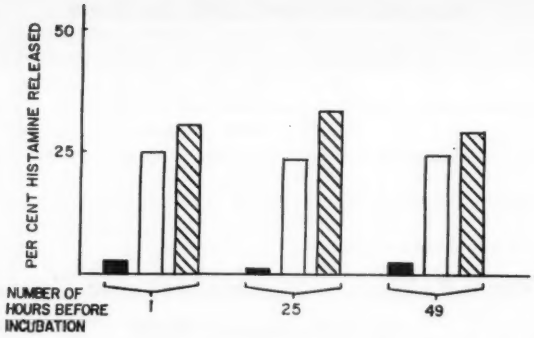

Fig. 1. Histamine release from chopped cat skin by compound 48/80 1, 25 and 49 hours after death of cat. ■: Controls; □: 48/80 10 μg/ml; ▨: 48/80 100 μg/ml. Duration of incubation, 30 min. Mean values of release in triplicate samples.

temperature, time, and some enzyme inhibitors on the histamine releasing activity of compound 48/80 and hence to learn something of the latter's mechanism of action.

Methods

The abdominal skin of a recently killed cat was shaved and stripped off. The subcutaneous fat was removed. The skin was chopped into shreds about 1 mm square. These were carefully mixed and washed with 50 ml warm incubation solution on a filter paper. They were then divided into portions weighing 1 g each. The samples were transferred to 25 ml beakers containing 5 ml incubation solution. The beakers were slowly rocked in a Warburg apparatus at 37° C. After 5 min the washing solutions were rapidly exchanged for 5 ml warm incubation solution containing different concentrations of histamine releaser, with the exception of the controls.

Incubation was carried out for 30 min, or sometimes 10 or 60 min, depending on the concentration of the releaser. The solutions containing the released histamine were heated to stop bacterial action and assayed on atropinized guinea-pig ileum. The histamine remaining in the minced tissue at the end of the experiment was released by boiling for 5 min in N hydrochloric acid and was similarly assayed following neutralization. Subsequently it was shown, by blocking the contractions with antihistamines, that the effect on the ileum was due to histamine.

The incubation solution contained sodium chloride 154 mmole/l, potassium chloride 2.7 mmole/l, calcium chloride 0.9 mmole/l, and Sörensen's phosphate buffer 10 per cent.

For investigation of agents blocking the action of 48/80, the experimental procedure was as follows. After washing, the skin fragments were incubated for 30 min in a solution containing the inhibitor. Thereafter 10 μg 48/80 was added and the incubation continued for another 30 min. The histamine released was tested in the usual way.

Unchopped skin could be used a few days after removal (Fig. 1). It was necessary to keep it in a moist chamber at 4° C.

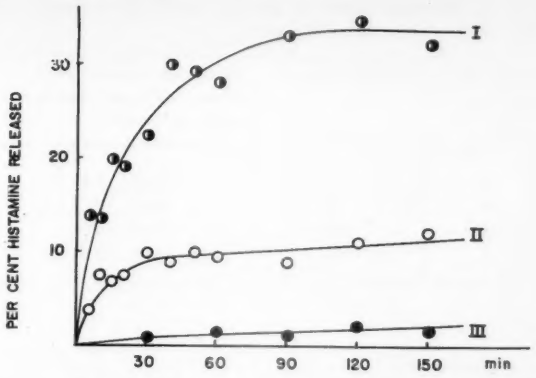

Fig. 2. Time course of histamine release from chopped cat skin by compound 48/80. I: 48/80 100 μg/ml; II: 48/80 10 μg/ml; III: controls.

Materials

Histamine liberator

Compound 48/80, prepared according to the method described by BALTZLY, BUCK DE BEER and WEBB (1949).

Blocking agents

Inhibitor from hip seeds. The inhibitor was prepared from air-dried ripe hips by a method described by HÖGBERG *et al.* (1956).

Allicin. The substance was prepared from garlic according to a technique reported by CAVALLITO and BAILEY (1944).

The other inhibitors, phenol, dinitrophenol, sodium tetrathionate and ninhydrin, were obtained from standard commercial sources.

Results

Histamine Content of Chopped Skin

The amount of histamine in the skin varied from one animal to another. In abdominal skin, between 2 and 25 μg/g tissue was found. The lowest values were obtained in young cats, a finding which accords with the observations made by RILEY and WEST (1953).

Spontaneous Release of Histamine

In the washing solution about 1 μg histamine or less per 5 ml was found and was demonstrable in the fluid within 10 min. In further washings only small amounts of 0.15—0.25 μg/5 ml were determined. Hence, only one washing was considered necessary.

The spontaneous release of histamine during incubation was very small, as shown in Fig. 2.

Table I. Histamine release in parallel samples from chopped cat skin. Time of incubation: 30 min

Number of samples	48/80 in μg/ml	Histamine released in % Mean value and standard error of mean
4	0	1.35 ± 0.21
4	10	8.85 ± 0.64
4	100	16.08 ± 0.89
4	500	25.03 ± 1.54

Reproducibility of Results

Table I shows the variation of histamine release in parallel samples from one animal and the reproducibility was considered satisfactory. The fact that assays on guinea-pig ileum may be associated with an error of about 10 per cent (AHLMARK 1945) could account for the variation. — The results reported in the following sections have been checked for statistical significance.

Effect of Time and Concentration

Fig. 2 shows the course of histamine release induced by different concentrations of 48/80. With 10 μg 48/80 per ml the liberation slowed down after 30 min, but with 100 μg/ml it continued for another hour. In the following experiments the most suitable duration, usually 30 min, was taken.

Table II. Influence of temperature on the action of 48/80 on chopped cat skin. Concentration of 48/80: 10 μg/ml. Duration of incubation: 10 min

Temperature	Histamine liberated in per cent the release at 37° C
0° C	0
10° C	0
20° C	0
25° C	25
30° C	100
35° C	100
40° C	100
45° C	40
50° C	0

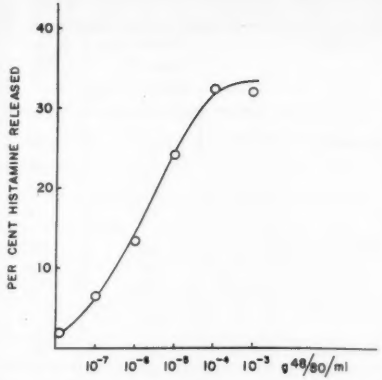

Fig. 3. Release of histamine from chopped cat skin by different concentrations of 48/80. Duration of incubation, 30 min. Mean values of release in triplicate samples.

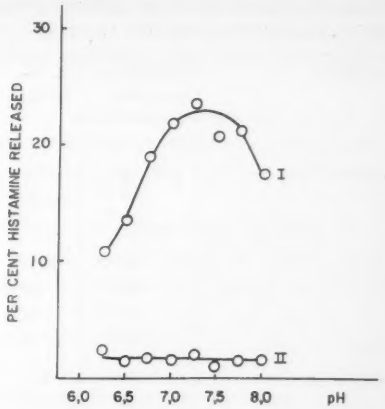

Fig. 4. Effect of pH upon histamine release from chopped cat skin by compound 48/80. I: 48/80 10 μg/ml; II: controls. Duration of incubation, 10 min. Mean values of release in triplicate samples.

Dose-Response Experiments

The release after incubation for 30 min with different concentrations of 48/80 is shown in Fig. 3. The threshold value was found to be 0.1 μg 48/80 per ml and 1 μg caused a significant release compared with the controls.

Action of Temperature

The influence of temperature is shown in Table II. With the doses used there was no distinct optimum, but the latter seems to be localized between 30° C and 40° C. Below 20° C and above 47.5° C no histamine was liberated. Heating to 50° C caused an irreversible inhibition of the effects of 48/80, while cooling to 0° C had no influence upon the subsequent incubation at 37° C.

Influence of pH

Incubation experiments at different pH were done with Sörensen's phosphate buffer. The proportion buffer to incubation solution was 1 : 9, in some experiments 1:4. When the initial pH was between 6.75 and 7.35 no significant pH changes were observed during the 30 min incubation. Below and above these values pH changes during incubation were obtained in spite of the buffer. Above pH 7.35 the changes were towards the acid side; below pH 6.75, towards the alkaline.

Table III. Influence of various inhibitors on histamine release from chopped cat skin by compound 48/80. Concentration of 48/80: 10 µg/ml

Inhibitor	Concentration of inhibitor in M	% inhibition	Inhibitor	Concentration of inhibitor in M	% inhibition
Ninhydrin	10^{-3}	100	Phenol	10^{-2}	80
	10^{-4}	40		10^{-3}	60
	10^{-5}	0		10^{-4}	45
				10^{-5}	0
Allicin	10^{-3}	100	Dinitrophenol	10^{-2}	100
	10^{-4}	80		10^{-3}	80
	10^{-5}	40		10^{-4}	50
	10^{-6}	0		10^{-5}	0
Hip seeds	2×10^{-3}	100	Sodium tetrathionate	10^{-3}	100
	10^{-3}	40		10^{-4}	50
	5×10^{-4}	0		10^{-5}	0

Fig. 4 shows the histamine release produced by 10 µg 48/80 per ml at pH values between 6.25 and 8.0. The optimum is not well defined but seems to be between pH 7.0 and 7.75. The following experiments were carried out at pH 7.25, where conditions were optimal and the pH changes were negligible.

Above pH 8.0 histamine liberation caused by 48/80 was still high, but in view of the substantial pH changes towards the acid side no conclusions were drawn concerning the activity of 48/80 at those pH levels.

Inhibition of Histamine Release Produced by 48/80

HÖGBERG and UVNÄS (1957, 1958, 1960) reported that some enzyme inhibitors blocked the disruptive action of 48/80 on mast cells in rat mesentery. Among the inhibitors were reagents fairly specific for protein amino groups and sulfhydryl groups.

In this study an amino group blocking agent, ninhydrin, was used and a sulfhydryl group blocking substance, allicin, was chosen. Each of them inhibited the histamine releasing activity of 48/80, as shown in Table III.

Various other enzyme inhibitors such as phenol, dinitrophenol, sodium tetrathionate and hip seeds were also found to be active (Table III).

Discussion

It has been shown that chopped cat skin can be used for testing the activity of compound 48/80. In preliminary experiments some other histamine-releasing substances, lecithinase A, extracts from *Ascaris lumbricoides* and *Cyanea capillata* were found to induce histamine liberation from the skin preparation.

The advantage of this method as compared with perfusion methods is that a large number of samples can be obtained from one animal for testing different concentrations of a compound or for examining the effects of different drugs.

Compared with the method described by MONGAR and SCHILD (1953), in which chopped guinea-pig lung was used, the skin method is more sensitive for testing compound 48/80; this because guinea-pig mast cells are less susceptible than cat mast cells to the action of 48/80.

The lowest dose of 48/80 that induces histamine release distinguishable from spontaneous liberation is about 0.1 μg per ml incubation fluid, though with some preparations the sensitivity is ten times less. Thus this method is almost as sensitive as the perfusion methods.

Not even very high doses of 48/80 served to release all the histamine present in the tissue. Either still higher doses are required or the skin contains a certain amount of non-releasable histamine, as was also pointed out by PERRY (1956).

The investigation showed that the effect of 48/80 is dependent upon time and concentration. The diffusion of liberator and histamine through the tissue is rapid enough to give a steep dose-response curve after incubation for 30 min; thus quantitative comparisons of activity are possible.

The action of compound 48/80 upon cat skin is dependent upon temperature and pH. The activity of 48/80 is irreversibly blocked by heating the skin to 50° C, and cooling to 0° C has no effect upon subsequent incubation at 37° C. There are also temperature and pH optima, though they were not well defined with the doses used. This suggests that the action of 48/80 upon cat skin depends on an enzyme system.

Other results lend support to this conclusion. Some enzyme inhibitors as the amino group reagent, ninhydrin, and the sulfhydryl group blocking substance, allicin, were found to inhibit the histamine releasing activity of 48/80 on chopped cat skin. The blocking action of dinitrophenol indicates that a phosphorylating process is involved in the release mechanism.

In a few experiments the action of 48/80 both on histamine liberation and on mast cell changes was studied, since the releasable histamine in the skin is thought to be localized in the mast cells (RILEY and WEST 1956). It was found that with release of histamine the mast cells lost their tingibility and disappeared. The effect is similar to that which BORÉUS (1960) has demonstrated in guinea pig, where histamine release and mast cell disappearance run parallel.

The results of this study thus accord with the working hypothesis of HÖGBERG and UVNÄS that compound 48/80 acts by means of an enzyme system attached to the mast cells.

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Acta physiol. scand. 1960. 50. 49—53

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Functional Localization as an Aid to Implantation of Permanent Electrodes into the Hypothalamus of Horned Goats

By

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Abstract

Andersson, B., N. Persson and L. Ström. *Functional localization as an aid to implantation of permanent electrodes into the hypothalamus of horned goats.* Acta physiol. scand. 1960. *50.* 49—53. — A method is described for implantation of permanent platinum iridium electrodes into the brain stem of horned goats. The final fixation of the electrodes is preceded by an acute stimulation experiment performed according to the technique of Hess. In this manner a satisfactory functional localization is secured.

When brain electrodes, which are later to be used for electrical stimulation of the unrestrained, awake animal, are implanted, two different techniques are usually applied. The electrodes may either be implanted by guidance of stereotaxic coordinates or Hess' technique (1932, 1949) may be followed. In the former case, the use of a stereotaxic instrument during the operation demands rather deep general anaesthesia. The stimulation experiment can be performed only after the electrodes have been permanently fixed in position and the animal has recovered completely from the anaesthesia. On the other hand, Hess' technique, or modifications of it, permit changes in the position of the electrodes during an acute stimulation experiment, which is advantageous if one wants to obtain a satisfactory functional localization.

The advantage of a good functional localization of electrodes for more permanent use is obvious. With this end in mind a method has been developed for implantation of permanent electrodes into the hypothalamus of horned goats, which is based on the result of a preceding acute stimulation experiment performed according to Hess' technique, modified for use in goats.

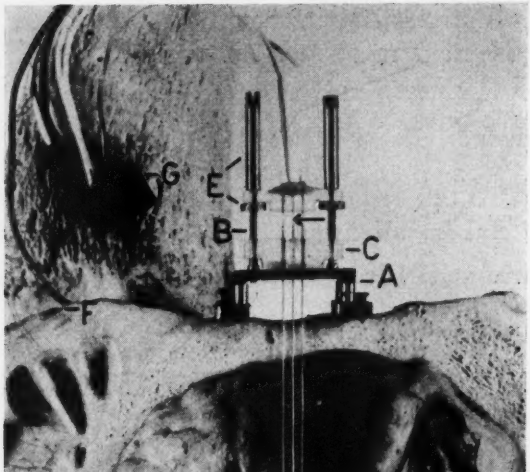

Fig. 1. A paramedian sagittal section through the cranium of a goat, to show how electrodes are temporarily fixed in the hypothalamus during the acute stimulation experiment.
A: 4-legged electrode holder screwed onto the skull.
B: Threaded guiding rods for the electrodes.
C: A guide with three parallel channels, just fitting the diameter of the electrodes.
D: Uninsulated tips of the electrodes.
E: Screw-nuts for temporary fixation of the electrodes on the guiding rods.
F: Indifferent electrode.
G: A contact for connection of the electrodes with the wires from the stimulator.
The arrow marks where the permanently fixed electrodes are cut prior to the removal of the complete electrode holder.

The acute stimulation experiment

Orientation on the skull. A day or two prior to the acute experiment, the tips of two sewing-needles were fixed as landmarks on the skull, as close to the dorsal midline as possible. X-ray pictures of the head were then taken from the side and from above. Guided by previous X-ray studies of sagittally split heads, the positions of the optic chiasma, the anterior commissure and the mammillary body were marked on the X-ray picture taken laterally. It was then possible to determine and mark on the X-ray pictures where the holder (Fig. 1, A) had to be placed in relation to the needle tips if a specific site in the hypothalamus was to be reached with the tips of the electrodes.

Operation. The operation for placing the electrodes in position was performed under local anaesthesia. A dorsal midline incision was made through the skin and the periost between and somewhat behind the horns, exposing the flat bone surface and the tips of the sewing-needles. The correct placement of the holder (A) was thus facilitated by the X-ray pictures showing the position of the tips of the sewing-needles in two planes and the direct viewing of these tips on the surface of the exposed bone of the skull. The legs of the electrode holder were secured to the skull with four screws. Three parallel channels for passage of the electrodes were then drilled through the bone using a burr guide temporarily fixed on the guiding rods (B) of the electrode holder. The burr guide was removed and replaced by an electrode guide of similar shape (C). Three platinum iridium electrodes (0.3 mm in diameter, Pt/Ir : 85/15) with uninsulated tips (1 mm) (D) were pushed gently through the openings of the electrode guide into the upper part of the area of the brain stem intended to be explored in the acute experiment. The electrodes were temporarily fixed to the guiding rods (B) by screw-nuts (E) and could later in the experiment be lowered step-wise by screwing the nuts downwards. An indifferent electrode (F) was screwed into the bone between the horns in front of the electrode holder and a contact (G) for connection of the electrodes with the wires from the stimulator was fixed on one horn.

Stimulation technique. The leads from the stimulator were attached to a balanced suspender in the roof over a pen in which the animals were allowed to move freely during the acute stimulation experiment. The resistance of each electrode *in situ* was within the range of 3 to 6 k Ω.

The stimulator used is of the constant-current type, thus having a very high internal resistance. It generates square pulses with a duration which can be varied from 0.5 to 10 msec. The pulse repetition frequency can be changed from 1 to 100 cps and the stimulus strength from 0.01 to 6 mA. As a direct current through the electrodes is likely to cause electrolysis at the electrode tips the output stage of the stimulator is transformer-coupled. Current feed back is introduced to increase the internal resistance. A device for measuring the electrode resistance during stimulation is included in the stimulator. The device consists of a cathode ray tube with vertical and horizontal amplifiers. One amplifier is connected to the stimulating electrode, giving a vertical deflection of the beam, proportional to the stimulus voltage (V). The other amplifier is connected to the feed-back resistor through which the stimulus current (I) flows, thus giving a horizontal deflection proportional to the current. The tangent of the deflection angle of the cathode spot is proportional to V/I, *i. e.* the electrode resistance. The gains of the two amplifiers can be varied to give a deflection angle of 45°. When the stimulus is turned off, a calibrated, variable resistor is substituted for the electrode resistance. This resistor is varied to give the same deflection angle as during stimulation, and the resistance is read off.

Fixation of the electrodes for permanent use

The electrodes were not permanently fixed to the skull bone until one or more of them had been lowered into a position where stimulation caused an effect considered worthy of a long term study. Three dental root screws were screwed into the bone surrounding the electrodes. The protruding heads of the dental screws and the place of intrusion of the electrodes were covered by a thick layer of dental cement which rigidly fixed the electrodes on the bone of the skull. The electrodes were then cut (at the arrow in Fig. 1). The complete holder and the contact on the horn were removed. The skin incision was sutured and leads with contacts at the free end were soldered to the cut ends of the electrodes. The contacts were fixed with adhesive tape onto one horn

Fig. 2. The head of a goat with implanted electrodes, shielded with an aluminium plate round the base of the horns. On the horn are contacts for connection of the electrodes with the wires from the stimulator.

and the lead from the indifferent electrode was attached to the other horn in the same manner (Fig. 2). An aluminium plate was bent round the base of the horns and was fixed in this position (Fig. 2). The dorsal opening of the aluminium shielding was covered with adhesive tape. In this way the electrodes were well protected from damage. Penicillium with protracted action was administered to the goats, which were then replaced into their previous environment.

Results and discussion

Thus far the method has been used for long term studies of the effect of stimulation of the preoptic "heat loss centre" and the hypothalamic "drinking centre" and for recording of electrical activity from these areas of the brain stem. The tips of electrodes implanted in this way have also been used as points of aim for proton irradiation. The results of these studies will be published separately.

Apparently not bothered by the presence of electrodes in the brain goats have been kept for more than six months showing a remarkable consistency in their response to stimulation. An example is given in Table I which illustrates the constancy of stimulus threshold for positive drinking effect in a goat with three electrodes placed in the hypothalamic "drinking centre".

Table I. Constancy of stimulus thresholds for positive drinking response in a goat with permanent electrodes implanted into the hypothalamic "drinking area"

Implantation of electrodes: Oct. 13th 1959

Date	A → O mA	B → O mA	C → O mA	A → B mA	B → C mA	A+B→O mA
1959 Nov. 23rd.....		0.3				
» » 30th.....		0.3	0.2		0.3	
» Dec. 1st.....		0.3				
» » 19th.....		0.3				
» » 21st.....	0.3					0.5
1960 Jan. 11th.....	0.2	0.3		0.15		0.4
» » 12th.....	0.2	0.3		0.15		0.4
» » 18th.....				0.2		
» » 20th.....				0.2		
» » 27th.....				0.15		0.5
» » 29th.....	0.2	0.4		0.2		0.4
« Febr. 5th.....	0.2	0.4		0.15		0.4
» » 7th.....				0.15		
» » 10th.....				0.2		0.5
» » 20th.....				0.2		
» » 23rd.....			0.2	0.15	0.3	
» » 26th.....			0.15	0.15		
» March 3rd.....	0.3		0.2	0.2		

The goat was kept in its normal environment during all tests. The effect was considered positive when drinking appeared within less than 10 secs of stimulation.

Temperature of the water accessible 10 to 12° C.

Stimulus: Pulse duration 3 msec. Frequency 50 cps. Electrode resistance 4 to 5 k Ω.

A, B and C: Hypothalamic electrodes — O: Indifferent electrode.

The stimulation threshold may, however, not reach such a consistency until a few weeks after the acute experiment. It has thus been observed that for some time after the operation there is a gradual increase in the minimal effective stimulus for obtaining a certain effect. The phenomenon may be due to slight foreign body reaction leading to gliosis or other structural changes around the tips of the electrodes. A tissue reaction of this kind is likely to dampen the effect of electrical stimulation so that stronger stimuli are needed to affect surrounding, reactive nervous tissue. The foreign body reaction has probably faded some weeks after the acute experiment, at which time the stimulus threshold starts to remain constant.

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Post-Stimulatory Electrical Activity in the Preoptic "Heat Loss Centre" Concomitant with Persistent Thermoregulatory Response

By

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Abstract

Andersson, B., N. Persson and L. Ström. *Post-stimulatory electrical activity in the preoptic "heat loss centre" concomitant with persistent thermoregulatory response.* Acta physiol. scand. 1960. *50.* 54—61. — Thermoregulatory response remaining after electrical stimulation of the preoptic "heat loss centre" was studied in nonanaesthetized goats. The intensity and the duration of the response was dependent upon the external and the internal temperatures of the animals. Recording of the electrical activity in the "heat loss centre" produced evidence that the response studied was in some way related to a long-lasting after-discharge recorded from the stimulated part of the brain stem.

The preoptic area has been considered the site of central thermoreceptors since Magoun *et al.* (1938) showed that diathermic warming of this part of the brain produces polypneic panting and sweating in the cat. Its importance as a "centre" regulating against hyperthermia was further demonstrated by lesion experiments showing that an inactivation of this area reduced or abolished the thermoregulatory response to body warming (Clark, Magoun and Ranson 1939). Additional information concerning the thermosensitivity of the anterior hypothalamus and the preoptic area was given by Folkow, Ström and Uvnäs (1949) using peripheral vasodilatation as an index of activation, and by von Euler (1950) who observed that during warming of the carotid blood a steady potential field developed between the supraoptic region and the rest of the brain. The preoptic "heat loss centre" has since been studied by electrical stimulation in unanaesthetized goats (Andersson, Grant and Larsson 1956, Andersson and Persson 1957). A striking phenomenon ob-

served in connection with electrical stimulation was the long persistence of the thermoregulatory effect. Providing the body temperature of the animals was not significantly reduced and the goats were kept at room temperature, their heat loss mechanisms remained activated for periods up to several minutes after cessation of stimulation. If the body temperature was subnormal, however, the duration of the after-effect was considerably reduced. It was suggested that the phenomenon might be due to a persistent after-discharge at some level of the supra-medullary brain stem (ANDERSSON *et al.* 1956). This hypothesis is supported by the results of the present investigation.

Methods

Four adult, female goats were used. Three permanent platinum-iridium electrodes were implanted into the preoptic area according to a technique recently described (ANDERSSON, PERSSON and STRÖM 1960). In the acute stimulation experiment, which preceded the permanent fixation of the electrodes, it was ascertained that at least one of the three points of stimulation was situated in the "heat loss centre". Each animal was then used for experiments during several months. For the chronic experiments the goats were kept collared in metabolism cages. For studies of the effect of a high environmental temperature the cages could be placed in an electrically heated chamber, where the temperature was kept constant at a desired level by means of a thermostat. Exposure to cold was obtained by placing the cages in a freezing room. A rapid fall of the body temperature of the goats was produced by giving cold water by stomach tube into the rumen.

Electrical stimulation: The main characteristics of the stimulus used was described earlier (ANDERSSON *et al.* 1960). Mainly unipolar stimulation was applied. The resistance with the electrodes *in situ* was within the range of 3 to 7 kΩ. The parameters of stimulation were: Strength 0.1—0.6 mA; Pulse width 3 msec; frequency 50 cps.

Recording technique: For recording of electrical activity in the preoptic area, a slightly modified, four channel "Mingograph" inkwriting electroencephalograph was used. The modification consisted of an addition of 1 MΩ resistors from the input grids to the ground. Without these resistors a grid current, large enough to stimulate, would flow through the electrodes when an input grid of the differential amplifier was left floating during the procedure of connecting the encephalograph to the electrodes. The encephalograph had a time constant of 0.1 sec and a rise time of 5 msec.

The differential amplifiers of the "Mingograph" were connected to record the potential differences between the electrodes (named A, B and C in rostro-caudal order). Usually the potential differences A to B, B to C and C to A were recorded. The fourth channel of the "Mingograph" was used for recording the respiratory rate, having its DC-input connected with a condensor manometer coupled to a spirometer. Ear surface temperature, as an index of peripheral blood flow, was recorded by thermocouples attached to the ear tips and connected to an "Ellab" galvanometer.

Results

The first goat used in this study was kept with two electrodes implanted into the preoptic "heat loss centre" from Nov. 24th 1958 to July 14th 1959. The temperature in the stable during this period gradually changed, having its

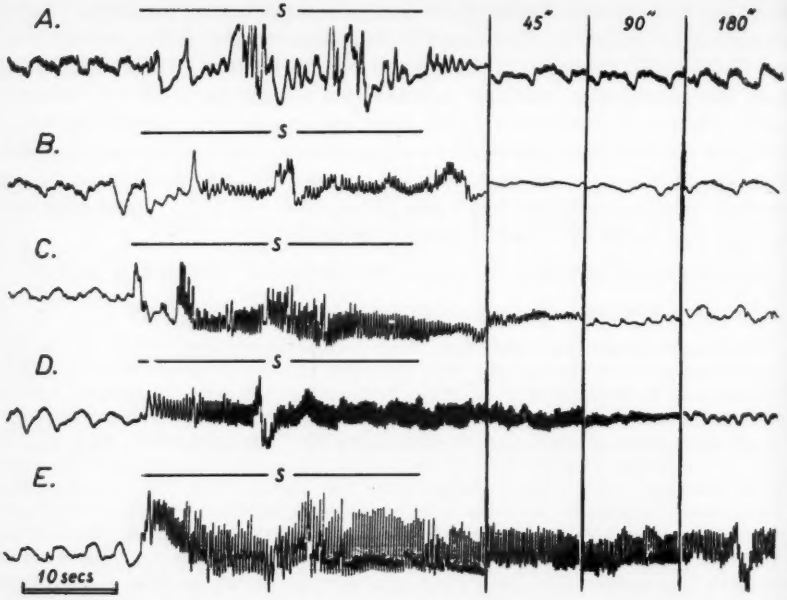

Fig. 1. Records of respiratory rate to show the influence of different external and internal temperatures on the intensity and the duration of the polypneic response to a constant electrical stimulus applied to the preoptic "heat loss centre" of a goat.
Record A: External temperature 15° C. Rectal temperature lowered from 38.8° C to 37.4° C by cold water administered into the rumen.
Record B: External temperature —5° C (Two hours, exposure). Rectal temp. 39.5° C.
Record C: External temp. 15° C. Rectal temp. 38.8° C.
Record D: External temp. 25° C. (Three hours' exposure.) Rectal temp. 39.0° C.
Record E: External temp. 33° C. (Three hours' exposure.) Rectal temp. 39.5° C.
S: Period of electrical stimulation. Stimulus : Strength 0.6 mA, Pulse width 3 msec, Frequency 50 cps., Duration 30 secs. Shivering is indicated by oscillations during inspiration in records A and B.

minimum of about 10° C at the beginning of Feb. and a maximum of about 25° C early in July. Concomitant with the rise in the environmental temperature, a gradual increase in the response of the "heat loss centre" to a constant electrical stimulus was observed. The increased response was manifested by a shorter latency period before onset of polypneic panting, and still more by prolonged duration of increased respiratory rate and peripheral vasodilatation after cessation of stimulation. For this reason the changes in response to a constant electrical stimulus at different environmental and body temperatures were analysed more closely in other goats.

Fig. 1 shows the influence of changes in environmental temperature and of a lowered body temperature on the respiratory response to a constant electrical stimulus in one of the animals. Fig. 2 illustrates the changes in vasodilatation

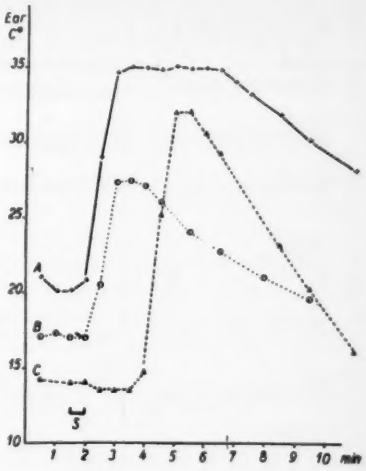

Fig. 2. Ear surface temperatures measured as an index of peripheral vasodilatation following upon a constant electrical stimulus applied to the "heat loss centre" under different thermal conditions.
Curve A: External temperature 15° C. Rectal temperature 38.9° C.
Curve B: External temperature 15° C. Rectal temperature lowered from 38.8° C to 37.5° C by cold water administered into the rumen.
Curve C: External temperature — 5° C (Two hours' exposure). Rectal temperature 39.6° C.
S: Period of electrical stimulation. Stimulus as in Fig. 1.

response under similar circumstances. The results were similar in other animals tested. It was thus found that two hours' exposure to cold (—5° C), although causing a slight increase in rectal temperature, reduced the respiratory response during the period of stimulation and shortened the polypneic after-effect considerably (Fig. 1 B). The latency period before the ear surface temperature started to rise increased to about two minutes and the period of peripheral vasodilatation ended somewhat earlier than after the same stimulus applied at 15° C (Fig. 2, curve C). The duration of peripheral vasodilatation was considered to be that interval in which the temperature of the ear surface started to rise, reached a maximum and just began to fall. Shivering was inhibited for about a minute and did not return to pre-stimulation intensity until the ear surface temperature had began to decline.

A lowering of the body temperature of the animals 1 to 2° C by cold water administered into the rumen reduced the thermoregulatory response to stimulation of the "heat loss centre" more conspiciously than did cold exposure. The polypnea was moderate during the period of stimulation and practically no respiratory after-effect was observed (Fig. 1 A). The period of peripheral vasodilatation was considerably shorter than that caused by the same stimulus at normal body temperature (Fig. 2, curve B) and shivering reappeared within 10 secs. and had reached pre-stimulation intensity within a minute after cessation of stimulation.

Three hours' exposure to a moderately increased environmental temperature (25° C) caused a slight rise in rectal temperature and resulted in a considerable prolongation of the polypneic after-effect (Fig. 1 D). The same period of exposure to a high environmental temperature (33° C), causing a rise in

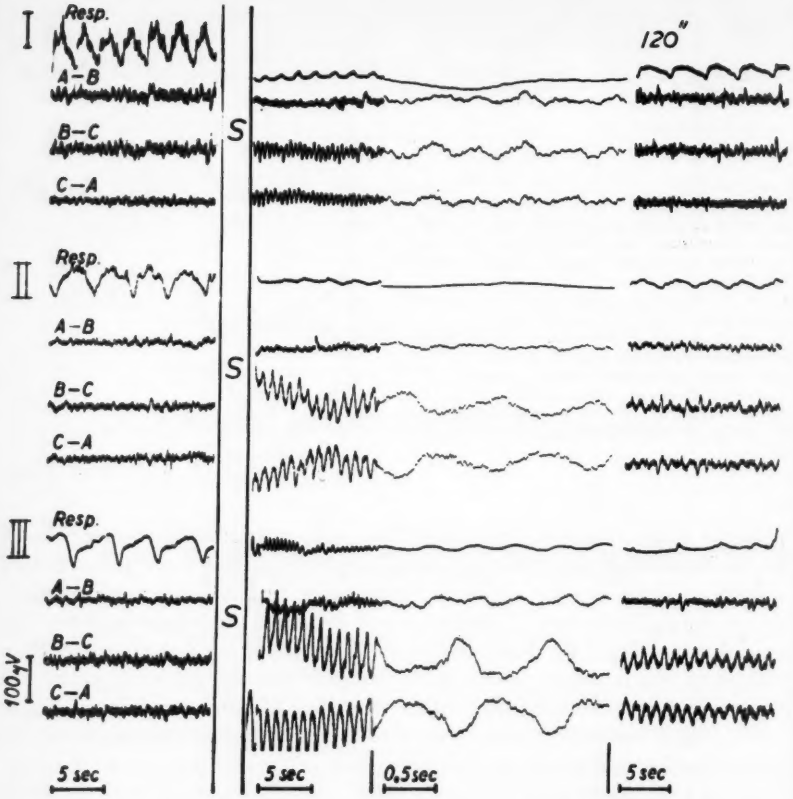

Fig. 3. Records of the respiratory rate (Resp.) and the potential differences between three electrodes implanted into the preoptic area of a goat, showing the influence of temperature changes on the persistent electrical activity recorded from the "heat loss centre" subsequent to electrical stimulation. The electrodes are named A, B and C in rostro-caudal order. Unipolar stimulation was applied via electrode C which was implanted into the "heat loss centre". The interval marked S indicates the period of electrical stimulation. Stimulus as in Fig. 1. The records were taken immediately before and after the period of electrical stimulation and 120 secs. after cessation of stimulation.
Record I: External temperature 15° C. Rectal temperature lowered from 38.9° C to 37.5° C by cold water administered into the rumen.
Record II: External temperature —5° C (Two hours' exposure). Rectal temperature 39.6° C.
Record III: External temperature 15° C. Rectal temperature 38.8° C.

rectal temperature of about 0.5° C, was even more efficient in increasing the polypneic response during and after stimulation (Fig. 1 E).

Peripheral vasodilatation appeared to be a more sensitive index of activation of the "heat loss centre" than was polypnea. Electrical stimulation, too weak to induce any respiratory effect, was thus often seen to cause a temporary rise of

ear surface temperature. The same phenomenon was observed by STRÖM (1950) using hypothalamic warming as stimulus to the "heat loss centre".

Recording of potential differences between the electrode tips before and immediately after stimulation revealed that unipolar stimulation via an electrode implanted into the "heat loss centre" caused a typical change in the electrical activity of the stimulated part of the brain stem. A stimulus, strong enough to cause a marked thermoregulatory response, gave rise to the appearence of regular slow waves on the electroencephalogram. This kind of activity lasted for up to four minutes after cessation of stimulation, during which time the amplitude of the waves gradually diminished. Provided an animal was kept in thermal balance in a constant environmental temperature, the recorded activity was directly proportional to the intensity of stimulation. The general character of the post-stimulatory electrical activity in a goat thermal balance, kept at 15° C is seen in Fig. 3, III. This figure also illustrates how post-stimulatory changes in the electroencephalogram were dependent on the thermal status of the experimental animal. Two hours' exposure to cold (—5° C) reduced the post-stimulatory activity moderately (Fig. 3, II). Although the frequency of the slow waves remained unchanged, their amplitude was diminished and they disappeared within two thirds of the time obtained subsequent to the same stimulus applied at an environmental temperature of 15° C.

Lowering the body 1 to 2° C caused a much more conspicuous reduction of the post-stimulatory electrical activity (Fig. 3, I). The amplitude and also the duration of the waves were markedly diminished and the recording regained its pre-stimulation appearence within half a minute after the end of stimulation.

Exposure to a high environmental temperature (33° C for two hours), however, did not significantly increase the post-stimulatory electrical activity as compared with the corresponding activity at 15° C.

In the experiment illustrated in Fig. 3, electrode A was situated slightly rostral to the "heat loss centre", as judged from X-ray pictures, and from the fact that stimulation via this electrode was not seen to activate any heat loss mechanisms. Stimulation via this electrode was not seen to change the pattern of the electroencephalogram. In other animals, stimuli applied via electrodes situated more caudally in the hypothalamus were often seen to evoke electrical activity, but of another character and of considerably shorter duration than that due to stimulation of the preoptic "heat loss centre".

Discussion

The main purpose of the present study was to find a possible explanation for the observation that heat loss mechanisms, evoked by electrical stimulation of the preoptic "heat loss centre", generally outlasts the duration of stimulation considerably (ANDERSSON *et al.* 1956). In addition the results obtained confirm earlier observations and seem to give some further information on the interac-

tion between peripheral and central thermoreceptors in the maintenance of thermal homeostasis. In the above mentioned experiments the site of central thermoreceptors (MAGOUN *et al.* 1938) was stimulated with a constant electrical stimulus at different external and internal temperatures. It was observed that cold exposure reduced the thermoregulatory response in spite of a slightly increased rectal temperature. However, a lowering of the body temperature with no change in environmental temperature was even more efficient in this respect. These observations support the concept that the preoptic thermoreceptors are inhibited both reflexly from surface cold receptors and more directly by a lowered temperature of the internal environment. Variations in degree of tissue damage surrounding the uninsulated tips of the electrodes cannot account for the observed changes in the response to a constant electrical stimulus. Previous experience with the method of implantation used and of the type of stimulus applied make such an explanation most unlikely. All experiments described here were performed in animals with the electrodes implanted for more than a month, a period of time which seems sufficient to stabilize the response to electrical stimuli (ANDERSSON *et al.* 1960).

When an adequate electrical stimulus was applied to the "heat loss centre", polypnea and peripheral vasodilatation were seen to continue for several minutes after stimulation. The same kind of persistent response was seen subsequent to electrical stimulation of the "heat loss centre" in the nonanaesthetized dog (ANDERSSON 1960). An explanation of the phenomenon may be that the electrical stimulus does induce long-lasting after-discharge at some supra-medullary level of the brain stem. The post-stimulatory electrical activity recorded from the "heat loss centre" for periods up to four minutes may indicate that the postulated after-discharge arises in the preoptic "heat loss centre" itself, since exposure to cold or a reduction of the body temperature were found to depress the persistent thermoregulatory response as well as the post-stimulatory electrical activity recorded from the "heat loss centre".

There is, however, a striking similarity between the post-stimulatory activity recorded here from the preoptic area and the long-lasting rhythmical activity elicited in the hippocampus by different stimuli; among them electrical stimulation in the septum lucidum and the preoptic area (GREEN and ARDUINI 1954). GREEN and ARDUINI found that the hippocampal wave pattern was spread via the fornix proper to the mammillary body and the habenulo-peduncular tract. A conduction of hippocampal activity also to the preoptic area seems possible since anatomical studies have revealed that this area receives fibres from the stria habenularis and the fornix (LE GROS CLARK 1938, NAUTA 1956). The possibility thus remains that electrical stimulation in the preoptic "heat loss centre" may reflexly induce a long-lasting activity in the hippocampal formation which is discharged back into the preoptic area and there contributes to keep heat loss mechanisms activited even after discontinuation of the electrical stimulation.

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Acta physiol. scand. 1960. 50. 62—71

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The Effect of Blood Volume Variations on the Pulse Rate in Supine and Upright Positions and During Exercise[1]

By

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Abstract

GULLBRING, B., A. HOLMGREN, T. SJÖSTRAND and T. STRANDELL. *The effect of blood volume variations on the pulse rate in supine and upright positions and during exercice.* Acta physiol. scand. 1960. *50.* 62—71. — The effect of bleeding of about 10 per cent of the blood volume and reinfusion of this quantity of blood seven days later was studied in six male subjects. The study includes determination of the pulse rate at rest in supine and upright positions and during exercise on a bicycle ergometer. The physical working capacity (PWC_{170}) was computed from the pulse rates during work. The pulse rate in upright position and during exercise increases and PWC_{170} decreases immediately after the bleeding. During the next few days PWC_{170} increases and the pulse rate in upright position and during exercise decreases, while the pulse rate in supine position appeared to increase. On the sixth day PWC_{170} was found to be higher and the pulse rate in supine and upright positions and during exercise to be lower than before the bleeding. Reinfusion of the blood seemed to produce a further moderate increase in PWC_{170}. The decrease in PWC_{170} after the bleeding corresponded, on the whole, to the average relation in different individuals between PWC_{170} and the total amount of hemoglobin. The respiratory rate during exercise increased after the bleeding and also showed the same tendency as the pulse rate did during exercise and in upright position during the next few days.

[1] A preliminary report of the investigation was presented at the 1958 National Meeting of the Swedish Medical Association.

Results presented in a series of articles appear to show the existence of an inverse relation between the total amount of hemoglobin (THb) or the blood volume, on the one hand, and the pulse rate in upright body position and during exercise respectively, on the other (KJELLBERG, RUDHE and SJÖSTRAND 1949 a, b, KJELLBERG *et al.* 1950, SJÖSTRAND 1953). Results have also been presented, which seem to show that physical training can cause an increase in THb and blood volume and proportionately the physical working capacity (HOLMGREN *et al.* 1960). Moreover, physical training has proved able to influence the peripheral regulation of the circulation whereby the work performed or the oxygen volume taken up per pulse beat is increased with unchanged blood volume and THb (HOLMGREN *et al.* 1957). In connection with these investigations it seemed of interest to study the effect of venesection and reinfusion of the blood withdrawn upon the regulation of the circulation, both at rest in supine and upright positions and during exercise.

Studies regarding the effect of removal of different volumes of blood on the pulse rate in recumbent position and during tilting have been carried out earlier by GREEN and METHANY (1947). They found a direct relation between the increase in the pulse rate in upright position and the quantity of blood withdrawn. In recumbent position the pulse rate showed a smaller increase per 100 ml removed blood when the quantity of blood withdrawn was small than by larger blood loss.

Studies have also been carried out earlier on the effect of blood donation on the physical working capacity by KARPOVICH and MILLMAN (1942), SPEALMAN *et al.* (1948) and BALKE *et al.* (1954). In the first communication the effect of blood donation was studied on work of brief duration and high intensity as well as work of long duration that required endurance. The endurance seemed to be affected by an ordinary blood donation for up to three weeks. On the other hand, the ability to perform work during approximately one minute did not seem to be affected thereby. SPEALMAN *et al.* observed, among other phenomena after blood donation, higher pulse values with high external temperatures in upright position, during tilting with the head up and during exercise on the bicycle ergometer. Infusion of albumin decreased the pulse rate under these conditions. BALKE *et al.* (1954) studied, among other aspects, the effect of blood donation on the pulse rate during exercise on a treadmill. They observed that immediately after withdrawal of 500 ml blood the pulse rate during a certain work load was increased. However, 48 to 72 hours thereafter it returned to the initial level and was lower than initially on the eighth or ninth day after the bleeding.

Methods

The pulse rate was determined by means of palpation of the radial artery after 10 min rest in supine position and after 8 min rest in upright position. During exercise the pulse rate was determined by auscultation.

The work tests were performed on a bicycle ergometer with stepwise increase of the work load every 6 min (SJÖSTRAND 1947, WAHLUND 1948). The pulse rate was determined after 2, 4 and 6 min on each work load and usually reached between 150 and 170 beats per min on the highest load (see Table II). The pulse rate at the end of each period was plotted against the work load, and a straight line was drawn through the last two or three points. The work at a pulse rate of 170 (PWC_{170}) was obtained by slight intra- or extrapolation and has been used as a relative measure of the physical working capacity. In this experimental study no correction of PWC_{170} has been done for lacking steady state of the pulse rate as in earlier clinical studies from this laboratory. A correction for lacking steady state in the present material had not affected any of the mean values presented.

The respiratory rate during work was determined by auscultation after 3 min work on each load.

The THb and the blood volume were determined according to the alveolar CO-method (SJÖSTRAND 1948) with minor modifications (see HOLMGREN *et al.* 1957). The hemoglobin concentration in finger blood was determined spectrophotometrically as oxyhemoglobin in alkaline solution (0.25 ml blood in 50 ml 0.04 per cent NH_4-solution) cf. SUNDERMAN *et al.* (1953).

The blood was withdrawn as for an ordinary blood transfusion into ACD solution as anticoagulant (solution B, USP XV), one part to four parts of blood and was stored for 7 days at + 4° C. The volume of the withdrawn blood was determined by weighing, with the specific weight of the blood assumed to be 1.053. After the storage period the blood was reinfused in the same subject.

Material

Six male physicians served as experimental subjects. Their most important anthropometric data are presented in Table I. All except B. I. took part in regular gymnastics up to one month before the investigation.

Table I. Some data concerning the test subjects and the venesection

Test Subject	Age Years	Height cm	Weight kg	Heart vol. ml.	Total Hb g	Blood vol. l	Blood loss	
							g	per cent of blood vol.
T.S.	51	179	81	1,175	825	6.4	689	10.2
A.H.	34	183	79	990	950	6.6	631	9.1
B.S.	28	183	81	840	960	7.3	563	7.3
T.S-1	26	182	65	910	740	5.5	574	9.9
B.I.	34	186	72	835	840	6.1	670	10.4
S.B.	29	174	67	715	785	5.7	538	9.0

Procedure

The experiments were arranged so that THb and blood volume determinations were repeated two successive days. In connexion with the first determination a work test with an orthostatic test was done. The following day about 10 per cent of the

Table II. Some physiological data before and after venesection and after reinfusion

Subject		Before venesection	After venesection			After reinfusion	
			1 hour	2—3 days	6 days	1 hour	1—2 days
T.S.	Pulse rate						
	Supine	62	50	52	50	58	58
	Standing	62	78	62	62	66	62
	300 kgm/min	—	—	—	—	—	—
	600 »	110	118	106	102	118	102
	900 »	142	156	136	134	144	134
	1,200 »	170	175	—	170	168	160
	PWC_{170}	1,200	1,070	[1]1,240	1,200	1,225	1,300
	Hb conc. g %	13.0		13.2	12.5	12.1	
A.H.	Pulse rate						
	Supine	54	58	63	56	48	58
	Standing	64	78	74	68	54	66
	300 kgm/min	82	84	86	80	74	76
	600 »	102	114	108	102	94	102
	900 »	126	148	140	130	118	128
	1,200 »	158	174	—	166	152	160
	PWC_{170}	1,310	1,150	[1]1,180	1,230	1,380	1,290
	Hb conc. g %	14.5			12.1		13.9
B.S.	Pulse rate						
	Supine	60	68	66	56	58	50
	Standing	76	88	84	68	66	62
	300 kgm/min	86	92	92	80	94	
	600 »	—	108	106	94	96	
	900 »	110	128	122	112	116	
	1 200 »	142	156	150	138	138	
	1,500 »	172	176	—	164	164	
	PWC_{170}	1,480	1,380	1,410	1,570	1,570	
	Hb conc. g %	13.2		14.1	13.2		14.1
T.S-1	Pulse rate						
	Supine	56	64	62	58	60	59
	Standing	76	faint	76	78	68	66
	300 kgm/min	85	98	92	95	90	86
	600 »	126	130	120	113	116	108
	900 »	160	162	146	140	136	132
	1,200 »	179	182	—	—	166	162
	PWC_{170}	1,025	1,000	[1]1,175	[1]1,225	1,250	1,280
	Hb conc. g %	13.4		13.0	13.1		12.9
B.I.	Pulse rate						
	Supine	68	68	85	70	80	
	Standing	90	120	99	88	91	
	300 kgm/min	114	112	116	103	112	
	600 »	131	140	140	130	130	
	900 »	166	172	—	—	158	

Table II. (cont.)

Subject		Before venesection	After venesection			After reinfusion	
			1 hour	2—3 days	6 days	1 hour	1—2 days
	1.200 kgm/min	—	—	—	—	—	
	PWC_{170}	940	880	[1]975	[1]1,050	1,030	
	Hb conc. g %	13.8		13.1	12.8	13.9	
S.B.	Pulse rate						
	Supine	85	88	85	80	70	72
	Standing	99	100	91	96	78	
	300 kgm/min	108	114	108	105	100	
	600 »	132	137	122	126	118	
	900 »	152	161	143	148	139	
	1,200 »	176	183	—	172	163	
	PWC_{170}	1,125	1,000	[1]1,250	1,180	1,290	
	Hb conc. g %	13,7		13.1	13.8		14.4

[1] Extrapolated from a pulse rate < 150 beats/min.

blood volume was withdrawn (Table I), and one hour later an orthostatic pulse test and work testing were carried out. Two or three days after the bleeding, orthostatic and work tests were again carried out. On the sixth day after the bleeding all experimental subjects performed new tests, and on the seventh day the blood quantity withdrawn was reinfused. About one hour later the tests were carried out and, in some cases, also on one of the next few days. On five of the subjects exercise tests in supine position were carried out between the tests in sitting position, once before and once after the blood donation.

Results

The results are reported in Table II and in Fig. 1 and 2.

The resting pulse rate showed no definite change immediately after the venesection. On the second to third days it was somewhat higher, after which it decreased. During the first three days after reinfusion the mean resting pulse rate was somewhat lower than before the bleeding. In one case a slight immediate increase of the resting pulse rate was observed in connection with the reinfusion.

The pulse rate in upright position showed appreciably more marked variations than the resting pulse. Immediately after blood donation the mean value for five of the subjects increased from 78 to 93 beats per min. The sixth subject (T. S-l) almost fainted, and the orthostatic test had therefore to be interrupted. The pulse rate in upright position almost returned to the initial level on the second and third days after the blood donation and was the same

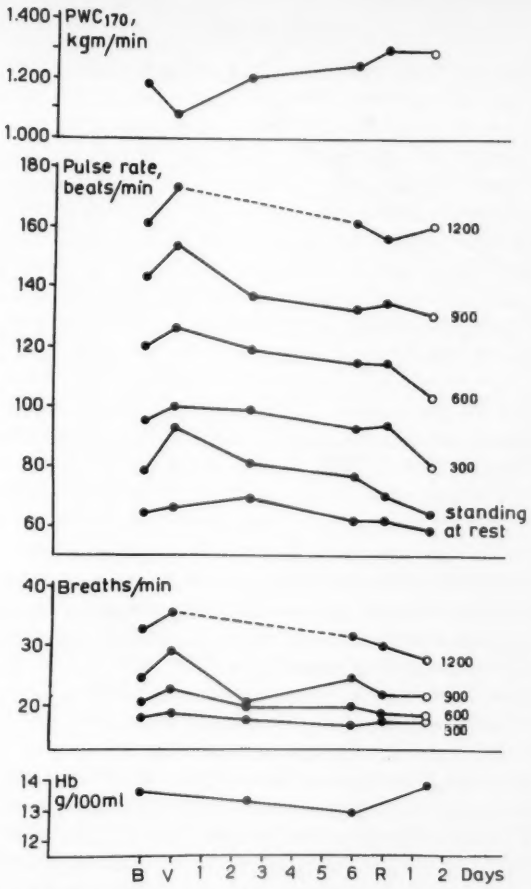

Fig. 1. From above: PWC_{170}, pulse rate during different work loads, in standing position and at rest, respiratory frequency during work and hemoglobin concentration. Data obtained before (B), 1 hour and up to 6 days after a blood loss (V) of about 10% of the blood volume, 1 hour and up to 2 days after reinfusion (R) of the withdrawn blood volume. The filled dots represent mean values on 4—6, open circles on 2—3 individuals.

as before the bleeding on the sixth day. After reinfusion the pulse rate decreased in upright position in four cases and increased somewhat in two.

The pulse rate during different work loads was higher immediately after the blood donation than before but decreased thereafter during the next few days.

The relation between the pulse rate at rest, in upright position and during

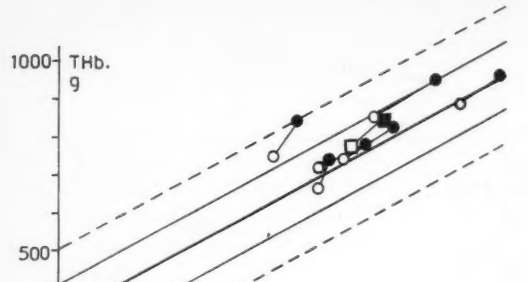

Fig. 2. Total hemoglobin in relation to PWC_{170} in six male subjects before (filled circles) and after (open circles) bleeding of about 10 per cent of the blood volume. Mean values are represented by squares. The lines represent the normal regression line and one respectively two times the standard error of estimate; values obtained from a study on 58 healthy subjects (Holmgren et al. 1957).

exercise is evident from Fig. 1. This shows that the variations in the pulse rate during heavier work loads, such as 900 kgm/min on the whole are the same as in upright position. During small loads the pulse curves present a course resembling the resting pulse curve.

The respiratory rate during exercise showed an average increase after the bleeding and thereafter a tendency to decrease as was the case with the pulse rate during exercise and in upright position (Fig. 1).

The physical working capacity determined from the pulse-work ratio, PWC_{170}, decreased in all subjects after the blood donation (range: 25—160 kgm/min). The mean decrease was 100 kgm/min, *i. e.*, 8.5 per cent. The reduction in PWC_{170} corresponded largely to the decrease in the THb according to the normal regression line between these parameters. (Fig. 2.)

After the immediate decrease of PWC_{170} it returned to the initial or a somewhat higher level on the second or third day. PWC_{170} showed an additional increase during the subsequent days and on the sixth day after bleeding the mean was 5.3 per cent (62 kgm/min) higher than before the withdrawal. Immediately after the reinfusion of the withdrawn blood PWC_{170} increased markedly in two of the experimental subjects, on an average for the six subjects 49 kgm/min or 3.9 per cent. In three subjects PWC_{170} was also determined one or two days later and did not show any definite variations.

The mean value of the hemoglobin concentrations decreased slightly after bleeding and did not reach the initial value before reinfusion was performed (Fig. 1).

Discussion

The investigation has shown that immediately after withdrawal of about 10 per cent of the total blood volume the pulse rate increases in upright position and during work while no sure increase is demonstrable during rest in supine position. During the first few days after the bleeding this increase in the pulse rate in upright position subsides, so on the sixth day the pulse rate becomes the same as before or lower. The pulse rate in supine position, conversely, shows a slight tendency to increase during the first few days after bleeding. The physical working capacity computed from the pulse response during exercise shows an 8.5 per cent decrease immediately after the venesection. By the second or third day after bleeding the working capacity has returned to its initial level and on the sixth day it was found to be slightly increased.

The observations on the pulse rate during work agree, on the whole, with those reported by BALKE *et al.* (1954). They do not appear, on the other hand, to confirm the views of KARPOVICH and MILLMAN (1940), *i. e.* that the physical working capacity should be reduced up to three weeks after a blood donation.

The present investigation demonstrates, as the report by BALKE *et al.* 1954, the effect of variations in the blood volume on the regulation of the blood circulation during changes in position and during exercise. The decrease in the physical working capacity after a blood donation corresponds, on the whole, to the decrease in the THb and the blood volume. At the same time, however, these studies show that this effect of bleeding is more quickly compensated than the restitution of the THb and apparently also of the blood volume. The working capacity per gramme hemoglobin, accordingly, increases during the days after the lowering of the blood volume. According to the values of the hemoglobin concentration, the working capacity per ml blood is also increased. This increase in the working capacity in relation to THb and blood volume is probably attributable to regulatory effects on the adaptation either of the peripheral blood circulation in the active musculature (see HOLMGREN *et al.* 1957 and 1959) or of vascular tonus with a change in the blood distribution between the central and the peripheral vessels whereby the central blood volume is restored.

This adaptation seems to persist even after the blood volume has been wholly or largely restored by an increase in the plasma volume which leads to an increase in the working capacity in excess of the initial level a week after the blood donation. Whether or not training as a result of repeated work tests contributed in the present experiments cannot be definitely determined. This might seem to be the most plausible explanation but it seems that BALKE *et al.* were able to preclude this possibility in their investigation, and they attributed the effect to an excessive dilution of the blood. However, the relative hemoglobin values in this study give no support for this suggestion.

Thus, bleeding appears to cause an immediate decrease in the physical working capacity and a compensation thereof within the next few days, probably primarily due to a vasomotor adjustment and to a dilution of the blood. The sum of these compensatory effects seems able to give rise to an increase in the physical working capacity to above the initial level. Subsequent reinfusion of the withdrawn blood seems able to produce a further increase in the working capacity and a decrease in the pulse rate in upright position but less than the opposite effects induced by removal of the same quantity of blood. The latter can be explained by a partial regression of the peripheral circulatory adaptation.

The behavior of the respiratory rate during exercise is interesting in several respects. An increase in the respiratory rate in relation to the oxygen uptake during work was also observed by BALKE *et al.* (1954), at least during heavier loads. There was a corresponding increase in the ventilation as well. The increase in the respiratory rate and ventilation parallel with the pulse rate increase indicates the existence of an intimate relation between the adaptation of the blood circulation and respiration.

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Effect of Training on Work Capacity, Total Hemoglobin, Blood Volume, Heart Volume and Pulse Rate in Recumbent and Upright Positions[1]

By

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Abstract

HOLMGREN, A., F. MOSSFELDT, T. SJÖSTRAND and G. STRÖM. *Effect of training on work capacity, total hemoglobin, blood volume, heart volume and pulse rate in recumbent and upright positions.* Acta physiol. scand. 1960. *50.* 72—83. — The effect of training, either with gymnastic exercises and running once or twice a week for several months, or with daily skiing in mountainous terrain for eight to ten days, has been studied on altogether 87 test subjects. Determinations were made of work capacity, expressed as work on a bicycle ergometer at a pulse rate of 170 and at relative steady state ($PWC_{170\,s}$), total amount of hemoglobin (THb) and blood volume, heart volume in recumbent position, and pulse rate at rest in recumbent and upright positions.

The *intermittent long-term training* was found to cause an increase in $PWC_{170\,s}$, THb and blood volume, and a decrease in pulse rate in recumbent position. The heart volume showed an insignificant average increase in two subjects groups, and decreased slightly in a group of middle-aged men. The relation between $PWC_{170\,s}$ and THb or blood volume corresponded, both before and after the training, to the relationship earlier demonstrated in a series of individuals differing with respect to age, body size, sex and degree of physical training. The *continuous short-term training* was also found to induce an average increase in the $PWC_{170\,s}$, blood volume and THb, and a decrease

[1] Preliminary reports of the present investigation have been given at the General Meeting of the Swedish Medical Association in December 1958 and 1959.

in the pulse rate in recumbent and upright positions. This type of training increased the PWC_{170} s more in relation to the THb and blood volume than intermittent long-term training did.

Some individuals showed no increase in THb. On the average these subjects showed a smaller increase in PWC_{170} s and a smaller decrease in the pulse rate in recumbent and upright positions than the individuals presenting an increase in THb. The effect of such ski training on PWC_{170} s and THb seems to be dependent upon the intensity rather than the daily duration of the training.

Observations were made on eight of the test subjects during a period of almost two years during which the intensity of the training was varied. The PWC_{170} s showed appreciable variation which was almost paralleled by the blood volume variation. The pulse rate in recumbent position varied inversely with the PWC_{170} s.

The results of the investigation indicate the existence of a direct relationship between work capacity and blood volume (total hemoglobin) under normal conditions.

Athletes with great physical work capacity, such as runners, skiers and bicyclists, are capable of a greater maximal oxygen uptake during exertion than other, otherwise comparable individuals. The greater capacity for oxygen uptake is accompanied by a greater so-called oxygen pulse, *i. e.* oxygen volume taken up per pulse beat, and that in turn by a greater capacity for muscular work, *e. g.* on a bicycle ergometer at a given pulse rate (WAHLUND 1948, and others).

Data have been presented indicating that the athletes' greater work capacity per pulse beat corresponds to a greater average blood volume and total amount of haemoglobin (KJELLBERG, RUDHE and SJÖSTRAND 1949 a, b). Accordingly, in relation to the total amount of haemoglobin (THb), athletes should on the average have the same work capacity, expressed as work load at a pulse rate of 170, as women and men without special physical training. The authors cited have also published results indicating that, in relation to heart volume, work capacity should be similar in athletes and other subjects.

The greater blood and heart volumes in well-trained individuals appear to be a direct result of the physical traning. Thus KJELLBERG *et al.* (1949 a, b) found that the blood volume and total haemoglobin in a small series of test subjects increased after one week's ski training in the mountains, and HOLMGREN *et al.* (1959) observed the same in a number of patients with neurosis who had undergone six weeks' systematic training.

Further support for this view has been given by WENNESLAND *et al.* (1959). They analyzed the variation in the blood volume in a series of individuals who differed with respect to bodily type and physical activity, and found a certain degree of variation with the latter factor. On the other hand, BASS *et al.* (1958) were unable to demonstrate an increase in the blood volume in a small series of test subjects who participated for three weeks in daily cross-country running and other organized exercise.

The present paper is a report of the results of an investigation of a large series of test subjects who participated in training of various types. The aim has been to provide further information on the effect of the training on the dimensions of the heart and the vascular system (blood volume) and to determine if such an effect can explain the increased work capacity demonstrable after physical training.

Material

The test subjects were hospital personnel (mainly physicians, nurses and laboratory assistants), medical students, wives of doctors and middle-aged men (business managers). All were healthy and had, according to their own opinion, an ordinary work capacity but were not specially trained at the onset of the investigation. Before the continuous training period all had taken part in gymnastics with exception for one male and one female. Observations were carried out on altogether 36 female and 51 male test subjects. A few of these have not been included in the final analyses of the findings because of incomplete data. Many of the test subjects participated in several test series.

Methods

The reader is referred to earlier publications from this department (HOLMGREN *et al.* 1957, 1959) for data on the laboratory methods. The work capacity was determined as the work load performed on a bicycle ergometer at a heart rate of 170 and in relative steady state (PWC_{170S}). The value was obtained from the linear pulse/work ratio observed during stepwise increasing loads, usually through a minor extrapolation or intrapolation.

The physical training consisted of 1) gymnastic exercises once or twice weekly for 30 to 40 min, 2) skiing in mountainous terrain, and 3) cross-country running alternating with gymnastic exercises (for 'managers'). The gymnastic exercises included training of muscle strength by a rapid succession of exhaustive exercises engaging different muscle groups, as well as training of the circulation and respiration by running and other rapid-tempo exercises engaging large muscle groups with a duration of several minutes.

The ski training took place at resorts located about 600 metres above sea level: in Februray and March at Storlien, in April at Vålådalen and in May at Riksgränsen. In addition one group ('physicians tour') participated in a long trip lasting eight days with about eight hours skiing daily and lodging at night in skishelters. The training in Storlien ('medical students') consisted of several hours skiing in groups and was not specially aimed at improving the circulatory and respiratory capacities. The training in Vålådalen and Riksgränsen, on the other hand, was planned in the first hand for circulatory conditioning with daily ski tours, usually lasting three to four hours, and almost daily five to ten kilometer exhaustive skiing on time. One female group ('tourists') did not undergo special training but participated daily in the skiing for eight to ten days at one of the aforementioned resorts.

Results

The observed data were divided into two main groups: one group including observations on the effects of intermittent training in the form of gymnastic exercises and cross-country running (Table I and II), and the other on the

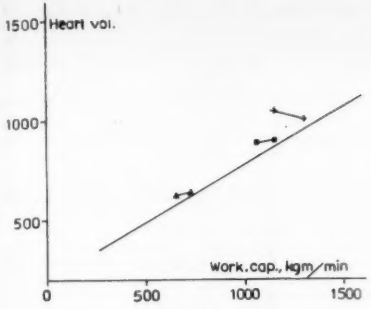

Fig. 1.

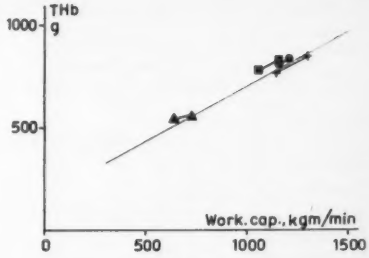

Fig. 2.

Fig. 1. The relationship between heart volume and PWC_{170} s before and after physical training once or twice weekly for two to four months. The symbols to the left indicate before and to the right after training.
▲ Female hosp.ass. ■ Physicians 1st period. × Business managers. Straight line drawn according to HOLMGREN et al. (1957).

Fig. 2. The relationship between THb and PWC_{170} s. The symbols to the left indicate before and to the right after training.
● Physicians' 2nd period. See Fig. 1 for identification of other symbols.

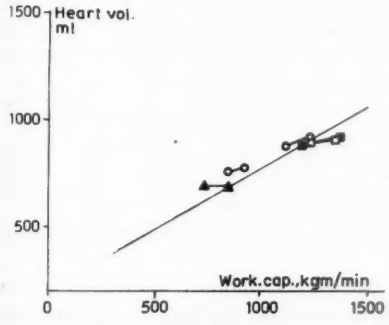

Fig. 3.

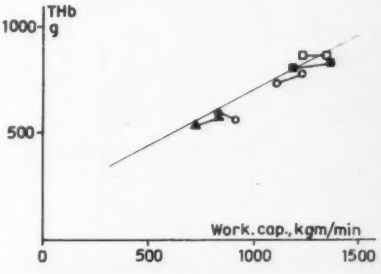

Fig. 4.

Fig. 3. The relationship between heart volume and PWC_{170} s before and after eight to ten days ski training in the mountains. The symbols to the left indicate before and to the right after training.
▲ Female hosp.ass. ○ Medical students, female (lower values), male (higher values). ■ Physicians. ⬚ 'Physicians tour'.

Fig. 4. The relationship between THb and PWC_{170} s before and after eight to ten days ski training in the mountains. Symbols to the left indicate before and to the right after training. See Fig. 1 and 3 for identification of symbols.

results of continuous training in the form of daily skiing (Table III and IV). The intermittent training lasted for two to three months (during the period October—April), or for three to four months with a break midways of about

Table I. Effect of training with gymnastics 1—2 times a week. Values before (b) and after (a)

Group	Sex	Age		n	PWC_{170}, kgm/min			Heart vol., ml		
		Mean	Range		b	a	diff. %	b	a	diff. %
Hosp. ass. 2–4 months	F	30	19—48	23	648.9	720.7	+ 11.1	624.8	634.0	+ 1.5
Physicians 2–4 months (1st period)	M	35	27—50	18	1,053	1,159	+ 10.1	889.6	901.5	+ 1.3
Physicians 2–3 months (2nd period)	M	34	27—51	13	1,153.5	1,209.6	+ 4.9	—	—	—
D:o 3 months additional ..	M	34	27—51	8	1,253	1,180	— 5.8	—	—	—
Managers ..	M	47	38—57	8	1,144	1,300	+ 13.4	1,056	1,003	— 5.3

Table II. Average differences between values after and before 2—4 months training with

	PWC_{170}, kgm/min			THb, g			Heart volume, ml		
	M	F	M + F	M	F	M + F	M	F	M + F
n	18	23	41	18	23	41	13	20	33
M_{diff}	+ 106	+ 72	+ 87	+36.8	+11.7	+22.7	+ 11.9	+ 9.3	+ 10.3
SD_{diff}	108	80	94	62.9	42.2	53.1	88	66	74
E_{diff}	25.5	16.7	14.7	14.8	8.8	8.3	24.4	14.8	12.9
t	4.2	4.2	5.92	2.48	1.33	2.74	0.488	0.628	0.798
p	< 0.001	< 0.001	< 0.001	0.02	0.2	0.01	0.65	0.5	0.4

Table III. Effect of ski-training 7—9 days. Values before (b) and after (a) training period

Group	Sex	Age, years		n	PWC_{170}, kgm/min			Heart vol., ml		
		Mean	Range		b	a	diff. %	b	a	diff. %
Hosp. ass.....	F	28	20—39	14	727	844	+ 16	690	683	— 1
Medical stud...	F	28	24—34	4	835	913	+ 9.4	754	774	+ 2.7
Tourists	F	32	24—38	5 (4)	830	890	+ 7.2	731	725	— 1
Physicians, training	M	36	27—52	20 (13)	1,188	1,374	+ 15.7	893.2	920.9	+ 3.1
Medical stud...	M	30	26—37	10	1,111	1,228	+ 10.5	875	916	+ 5.2
'Physicians tour'	M	36	30—43	7	1,233	1,351	+ 9.6	896	911	+ 1.7

training period

THb, g			Hb conc., g/100 ml			Blood vol., l			Pulse rate, recumbent			Pulse rate, upright 8 min.		
b	a	diff. %	b	a	diff. %	b	a	diff. %	b	a	diff. %	b	a	diff. %
543.9	555.6	+ 2.1	12.2	11.95	− 2.2	4.45	4.66	+ 4.7	71.3	65.7	− 7.9	83.6	84.2	+ 0.7
783.3	820.1	+ 4.7	13.9	13.7	− 1.2	5.62	5.98	+ 6.4	66.5	62.6	− 5.9	—	—	—
815.0	833.5	+ 2.3	13.8	13.6	− 1.4	5.90	6.12	+ 3.7	65.2	64.6	− 1.1	82.0	80.3	− 2.1
836	832	± 0	13.7	14.0	+ 2.2	6.10	5.94	− 2.6	64.5	68.0	+ 5.4	78.6	81.8	+ 4.3
770	853	+10.7	13.6	13.0	− 4.4	5.44	6.34	+16.5	60	55	− 8.3	79	71	−10.1

gymnastics 1 to 2 times a week of groups 'Hosp. ass.' (F) and 'Physicians 1st period' (M)

Hb conc., g/100 ml			Blood volume, l			Pulse rate, recumbent			Pulse rate, upright		
M	F	M+F	M	F	M+F	M	F	M+F	M	F	M+F
18	23	41	18	23	41	18	23	41	8	12	20
− 0.17	− 0.27	− 0.22	+ 0.36	+ 0.21	+ 0.28	−3.9	−5.6	−4.9	−3.5	−0.5	−1.7
0.70	0.61	0.64	0.47	0.37	0.42	4.61	7.63	6.46	9.4	10.9	10.2
0.165	0.127	0.100	0.111	0.077	0.066	1.09	1.59	1.01	3.32	3.15	2.28
1.03	2.13	2.20	3.24	2.73	4.24	3.58	3.52	4.85	1.05	0.16	0.75
0.3	0.05	0.03	0.01	0.01	< 0.001	0.001	0.001	< 0.001	0.35	0.9	0.5

THb, g			Hb conc., g/100 ml			Blood vol., l			Pulse rate, recumbent			Pulse rate, upright		
b	a	diff. %	b	a	diff. %	b	a	diff. %	b	a	diff. %	b	a	diff. %
549.3	580.4	+ 5.6	11.9	11.5	−2.6	4.63	5.08	+9.5	68.6	64.0	−6.8	84.9	78.2	−8.9
589	566	− 3.9	13.5	12.9	−4.4	4.35	4.38	—	66	61	−7.6	77	75	—
555	563	+ 1.5	12.1	12.2	—	4.59	4.65	+1.3	67	66	—	—	—	—
810.4	837.1	+ 3.3	13.43	13.17	−2	6.03	6.28	+4.2	65.4	63.1	−3.5	75.5	71.3	−5.6
743	782	+ 5.2	13.7	13.5	−1.5	5.42	5.79	+5.8	65	64	—	84	81	−3.6
878	869	− 1	14.1	13.4	−5	6.23	6.50	+4.3	68	64	−6	84	77	−8.3

Table IV. Average differences between values after and before ski-training of groups 'Hosp. ass.' (F)

	PWC_{170}, kgm/min			THb, g			Heart volume, ml		
	M	F	M+F	M	F	M+F	M	F	M+F
n	19	14	33	20	14	34	17	10	27
M_{diff}	+181	+117	+154	+26.7	+31.1	+28.5	+27.7	−7.0	+9.3
SD_{diff}	79.6	85.1	86.8	43.5	57.3	48.9	120	43	99
E_{diff}	18.3	22.8	15.1	9.73	15.3	8.39	29.1	13.6	19.0
t	9.9	5.14	10.2	2.74	2.03	3.40	0.95	0.51	0.49
p	0.001	0.001	0.001	0.01	0.05	0.001	0.4	0.6	0.65

one month (Christmas holidays). Some of the test subjects participated in training two to three months in both 1957 and 1958. The results from the two periods have been treated separately. The larger group of first-time trainees was made up of test subjects who started the training at different times during the period from 1957 to 1959. The group of business managers underwent training from April to June 1958.

Intermittent training. Table I gives the mean values before and after training, in the different intermittently trained groups, for $PWC_{170\ s}$, pulse rate, THb, blood volume and haemoglobin concentration (in peripheral blood). Table II shows the dispersion of the values and the degree of significance of a training effect for the larger male and female intermittent-training groups. It is evident from Table I that the different groups (except for a period of additional training during 3 months) consistently showed an increase in $PWC_{170\ s}$, THb and blood volume and a decrease in the resting pulse rate. Table II shows that after the training these changes are significant for the two larger groups. In two groups the heart volume showed an average increase, although not significant; in the 'managers' group an average decrease was observed. A group of subjects took part in a second intermittent training period but then only minor average changes were obtained. This group showed a greater work capacity and blood volume at the start of the second training period than before the first period.

The relation between the variations in $PWC_{170\ s}$, and THb and heart volume respectively, is apparent from Fig. 1 and 2. With the exception of the heart volume in the 'managers', the values mainly fall along the regression line earlier calculated for a material of healthy children, women, men in ordinary training, and well-trained athletes (HOLMGREN *et al.* 1957). The managers have on the average larger hearts before training than expected from THb and this relationship is normalized by the training. In this group the $PWC_{170\ s}$ varies before and after training with THb and not with the heart volume.

Continuous training. The results of corresponding examinations before and

and 'Physicians', training (M)

Hb conc. g/100 ml			Blood volume, l			Pulse rate, recumbent			Pulse rate, upright		
M	F	M+F	M	F	M+F	M	F	M+F	M	F	M+F
20	14	0.34	20	14	34	19	14	33	13	14	27
−0.26	−0.44	−0.34	+0.25	+0.45	+0.37	−2.3	−4.6	−3.3	−4.2	−7.6	−6.0
0.73	1.10	0.90	0.445	0.721	0.573	8.17	7.90	8.01	13.1	8.4	10.8
0.163	0.294	0.154	0.102	0.193	0.098	1.87	2.11	1.40	3.63	2.23	2.08
1.595	1.497	2.208	2.50	2.34	3.78	1.230	2.180	2.357	1.157	3.41	2.885
0.15	0.15	0.05	0.02	0.04	0.001	0.2	0.05	0.02	0.3	0.01	0.01

after a shorter period of continuous training are presented in Table III and IV. These show that $PWC_{170\ s}$ increased significantly, as did the blood volume and THb, while the pulse rate in recumbent and upright positions decreased significantly for the systematically trained groups. The heart volume increased slightly but not significantly.

Fig. 3 and 4 show the relation between $PWC_{170\ s}$, and THb and heart volume respectively, before and after the training. It is apparent that $PWC_{170\ s}$ has increased more than was anticipated on the basis of the estimated relation for different individuals between $PWC_{170\ s}$ and THb and heart volume respectively. The blood volume may be seen to have increased more than THb, a fact which only partly might explain the increase in $PWC_{170\ s}$, however.

Eight of the male test subjects were examined repeatedly during the period from October 1957 to June 1959. All participated in ski training in both 1958 and 1959. Data from these subjects show the variation of $PWC_{170\ s}$, blood volume, and pulse rate in recumbent position (Fig. 5). In the preparation of the data for presentation in the diagram in Fig. 5, minor adjustments of the time axis were made for the comparison of the observations in different individuals as the laboratory determinations were not always carried out simultaneously, and as the date of the mountain training period varied somewhat for the different subjects. The diagram shows a direct relation between $PWC_{170\ s}$ and blood volume and an inverse relation between these data and the resting pulse rate. There was a mean increase in $PWC_{170\ s}$ from 1,081 kgm/min to a maximum of 1,419 kgm/min after mountain training and a concurrent increase in blood volume from 5.43 to 6.08 litres, while the resting pulse rate decreased from 70.3 to 61.5 beats per minute. Neither $PWC_{170\ s}$ nor the other values returned completely to the initial levels during the interval between the periods of physical training.

The effect of the continuous training varied somewhat in the different groups. The greatest increase in $PWC_{170\ s}$, approximately 16 per cent, was obtained in the groups trained more systematically while staying at resorts. The 'tourist' group, the 'medical students' and the physicians who participated

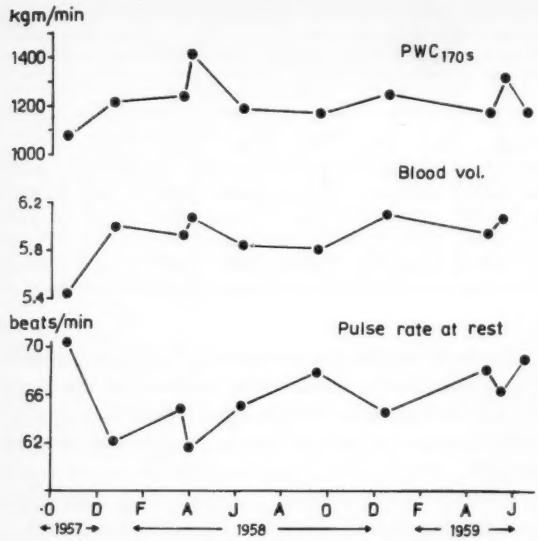

Fig. 5. Average variations of PWC_{170S}, blood volume and pulse rate at rest in recumbent position in eight of the test persons observed from October 1957 to June 1959. Intermittent training October—March, ski training 8 days in March 1958 and May 1959, no special training June—September.

in the ski tour showed an average increase of about 9 per cent. The physicians taking the tour showed largely unchanged THb but, on the other hand, a 5 per cent decrease in haemoglobin concentration and a corresponding estimated increase in blood volume.

Comparison of the data after ski training for those subjects in the 'hospital assistants', 'physicians' and 'physicians tour' groups, who did not show an increase in THb, with the data from the other subjects in these groups shows that there were mean differences between these two series in other respects as well (Table V). The first series (constant THb) presented a smaller increase in $PWC_{170\ s}$ and a smaller decrease in pulse rates in recumbent and upright positions than the second series (increased THb). However, the material is small and the number of males and females, respectively subjects taking part in more systematic training and in ski tour, varies in the groups. The present material is therefore not suitable for a statistical treatment and allows no definite conclusions in this respect.

Discussion

The present investigation shows that prolonged intermittent training causes an increase in $PWC_{170\ s}$ which corresponds to the increase in blood volume, *i. e.* the effective volume of the vascular system. The heart volume, as well, showed a mean increase in most of the groups but the individual variations were greater and the differences were not statistically significant. This might

Table V. Comparison between individuals with (A) respectively without (B) an increase of the THb after ski-training

Values before (b) and after (a) training

	PWC_{170}, kgm/min			Blood vol., l			Pulse rate recumbent			Pulse rate upright		
	n	b	a	n	b	a	n	b	a	n	b	a
A	23	930	1,089	20	5.20	5.71	23	66.1	61.9	21	80.5	74.0
B	13	1,135	1,271	11	5.86	5.87	13	68.9	67.7	11	82.8	80.3

be due to the fact that the heart volume does not, or to a less degree, decrease after a long period of extensive physical training (HOLMGREN and STRANDELL, 1959). The group of managers includes some earlier especially well-trained subjects and shows on the average large hearts before the training and practically unchanged heart volumes after the training period. The average heart volume of this group comes closer to the regression line for ordinary subjects after training, see fig. 1.

It was further found that $PWC_{170\ s}$ and blood volume increased after continuous, short-term training (ski training in the mountains) but not in the same proportion to each other as after intermittent long-term training. The work capacity per litre of blood volume therefore may increase after intensive training of short duration. Thus, for the larger groups of male and female test subjects $PWC_{170\ s}$ increased by 310 kgm/min per one litre of blood volume with intermittent, long-term training but by 420 kgm/min with the ski training.

The relationship between $PWC_{170\ s}$ and the blood volume can be attributed to a causal connection. The stroke volume of the heart during exercise has been found to be related directly to the blood volume (SJÖSTRAND 1956, HOLMGREN, JONSSON and SJÖSTRAND 1960), comprising on an average 2 per cent of the blood volume. In these investigations it was also shown that an increase in the $PWC_{170\ s}$ of 290 kgm/min corresponds to an increase of 20 ml in the stroke volume of each ventricle, and therefore to an increase of one litre in the blood volume. These computations demonstrate the intimate relation which exists between the blood volume (effective volume of the vascular system) and the functional capacity of the heart during exercise, in comparisons between different individuals as well as within the individual in different states of training.

After continuous, short-term training the $PWC_{170\ s}$ is found to increase more than corresponding to the increases of heart volume and blood volume. This might be due to a more efficient peripheral circulatory adaptation to muscular work, resulting in a larger utilization of the blood oxygen than before the training.

BASS *et al.* (1958) were of opinion that they could not confirm the earlier observation (KJELLBERG *et al.* 1948) that the blood volume may increase in connection with physical training. The reproducibility of the method used by BASS *et al.* for blood volume determinations proved so low, however, (in one of five test subjects in the control material the blood volume decreased by 1.65 l, *i. e.* 25 per cent, during the observation period) that definite demonstration of a variation of the order of magnitude in question here could not be expected in the test series of these authors. Nor did they give any measure of the work capacity, thereby making evaluation of the effect of the training impossible. The contention of BASS *et al.* that the carbon monoxide method for determining the blood volume is not suitable for use in conjunction with training because carbon monoxide is taken up by the myoglobin is contradicted by the computations of SJÖSTRAND (1948), and by a comparison with other methods for blood volume determination (WIKLANDER 1956, essentially confirmed by BIRKE *et al.* 1960). The observations reported here agree also with data on the acute effect of blood withdrawal on work capacity (GULLBRING *et al.* 1960). The blood withdrawal was found to decrease $PWC_{170\,s}$ in largely the same proportion to the blood quantity as training proved able to increase it.

Of interest for continued investigations of physical training is the observation that the effect on both the work capacity and the blood volume appears to vary with different training methods. Physical exercise of long daily duration but moderate intensity, such as a skiing tour, seems to have less effect in this respect than training of shorter daily duration with varying tempo up to exhaustion. This also appears to agree with the general experience of athletes.

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Acta physiol. scand. 1960. 50. 84—87

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Intracellular Localization of Dopamine in Cow Intestine

By

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Abstract

BERTLER, Å., N.-Å. HILLARP and E. ROSENGREN. *Intracellular localization of dopamine in cow intestine.* Acta physiol. scand. 1960. *50.* 84—87. — In previous investigations a certain type of chromaffin cells containing dopamine has been demonstrated to occur in ruminant tissues (FALCK, HILLARP and TORP 1959, BERTLER *et al.* 1959). It seemed likely that dopamine in these cells was hold in intracellular particles in a similar manner as noradrenaline and adrenaline in the adrenal medulla (HILLARP, LAGERSTEDT and NILSON 1953). In this study the intracellular distribution of dopamine in the cow intestine which contains high amounts of this amine, has been studied. Some factors affecting this distribution have also been included.

Experimental

The duodenum from cow was obtained from the slaughter house within 30 minutes after death of the animal. It was washed in ice-cooled 0.9 per cent sodium chloride solution and transported to the laboratory on ice. The mucosal layer was stripped off and homogenized in about 4—5 times its weight of 0.3 M sucrose solution. The homogenate was centrifuged at 800 × *g* for 6 min. The supernatant was sucked off. The residue, which in the following is called low speed sediment, was extracted with 0.4 N perchloric acid. The supernatant was centrifuged at 26,000—38,000 × *g* for 40 min. To the supernatant (high speed supernatant) 4 N perchlorid acid was added to give a final concentration of 0.4 N. The sediment (high speed sediment) was extracted with 0.4 N perchlorid acid. The dopamine and 5-hydroxytryptamine contents of the three fractions were estimated as described earlier (BERTLER, CARLSSON and ROSENGREN 1958, CARLSSON and WALDECK 1958, BERTLER and ROSENGREN 1959). The results are given in Fig. 1.

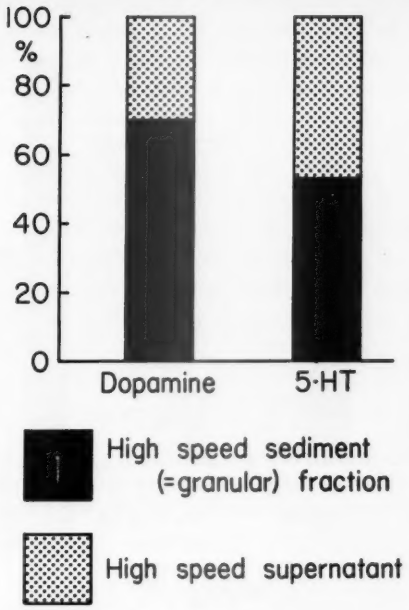

Fig. 1. *Distribution of dopamine and 5-HT in sediment and supernatant after high speed centrifugation of low speed supernatant.* The figures stand for per cent of the amount of 5-HT or dopamine present in the low speed supernatant.

The release of these amines from the granules has been studied in othe experiments. The high speed sediment was suspended in 5—6 ml 0.3 M sucrose. I qual volumes of the suspension were then treated in different ways. One ml was mixed with 5 ml of the following solutions: 0.5 M sodium acetate buffer, pH 4.0, 0.3 M sodium acetate buffer, pH 6.0, and redistilled water. The mixtures were left at 0° C for 15 min. Another ml was frozen and thawed 5 times. Then it was mixed with 5 ml 0.3 M sucrose. One ml of the suspension in 5 ml 0.3 M sucrose served as control. After centrifugation the sediments were examined for their amine contents as described above. The results of a typical experiment are found in Fig. 2.

All the steps of preparation of the fractions were carried out at 0° C.

Results and discussion

The data in Fig. 1 show that 70 per cent of the dopamine present in the low speed supernatant was recovered in the high speed sediment. The corresponding figure for 5-HT was slightly above 50 per cent. This sediment is mainly made up of mitochondria, but may also contain other particles. Thus in similar fractionation of the adrenal medulla the cathechol amine containing granules are found in this fraction. It is possible that the two amines are situated

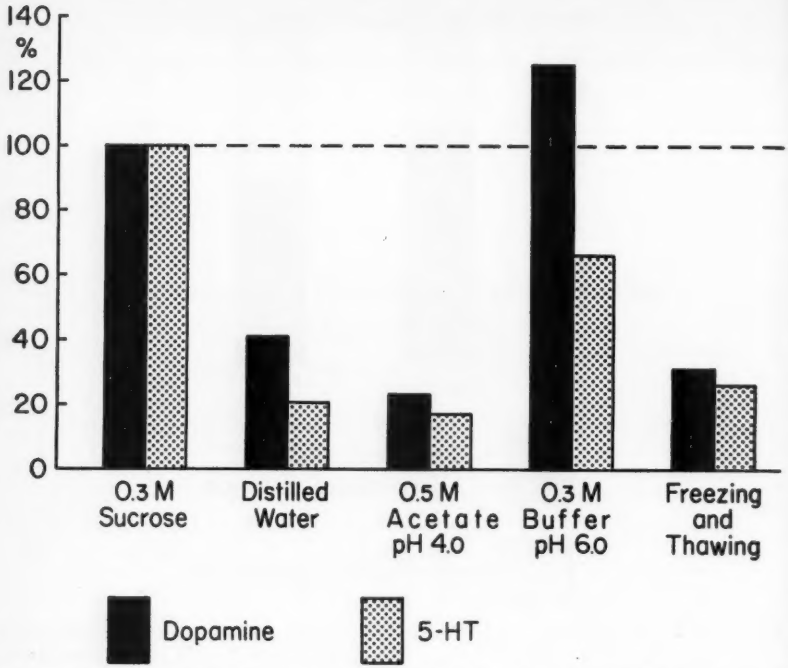

Fig. 2. *Release of 5-HT and dopamine from the granular fraction under various conditions.* Ordinate: amount of amine in granules expressed as per cent of control (0.3 M sucrose).

in a similar type of particles, but this has not been investigated as yet. The amounts of dopamine in the low speed supernatant represents only about one tenth of the total content of dopamine present in the original homogenates. The corresponding values for 5-HT was about one fifth. These relatively low figures may be due to that only a small percentage of the cells were broken, as the homogenization was very gentle in order to prevent destruction of the intracellular particles. That it was so was supported by the fact that after the similar homogenization technique on rabbit intestinal mucosa most of monoamine oxidase generally known to be located in the mitochondrial fraction was found in the low speed sediment and showed the same distribution pattern as 5-HT. The difference in the yield of dopamine and 5-HT at homogenization may be due to a difference in the fragility of the two types of cells in which the two amines are localized.

As can be seen in Fig. 2 most of the dopamine was liberated from the particles by suspending these in distilled water or a 0.5 M buffer solution of pH 4.0. Disruption of the granules by freezing also released most of the amine content

of the particles. After suspension in 0.3 M buffer solution of pH 6.0 essentially all of the dopamine was found in the particles. In these experiments 5-HT behaved in a similar way as dopamine. The observations on the intracellular localization of 5-HT found here were in agreement with the results obtained by BLASCHKO (1956) and BAKER (1958, 1959) from dog intestine.

In a study carried out by SCHÜMANN (1958) on the localization of dopamine in the splenic nerves of the cow evidence was presented that this amine was not bound to granules. According to this author dopamine was located in the cytoplasm and only was an intermediate in the noradrenaline formation. The results of the present investigation indicated however that the dopamine in the peripheral tissues of ruminants was contained in intracellular particles. The binding mechanism of dopamine appeared to have certain similarities to that of noradrenaline and adrenaline in adrenal medulla; the amines being released from the particles under the same conditions (HILLARP and NILSON 1954).

This work has been supported by grants from the Swedish Medical Research Council, by the Air Force Office of Scientific Research of the Air Research and Development Command, United States Air Force, and Medical Faculty, Lund.

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Acta physiol. scand. 1960. 50. 88—94

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Oxygen Intake and Thermal Balance in Naked Young Men during Rest and Sleep at Various Ambient Temperatures

By

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Abstract

Andersen, K. L. and B. Hellström. *Oxygen intake and thermal balance in naked young men during rest and sleep at various ambient temperatures.* Acta physiol. scand. 1960. *50.* 88—94. — The metabolic and thermal responses of three young men were investigated when resting or sleeping naked for eight hours at various ambient temperatures between 30 and 20° C. The results revealed that the naked men have a rather narrow thermal comfort zone. Exposure to 30 °C was felt to be uncomfortably warm. At 20° C two of the three subjects gave up after four hours exposure because they were too cold. The oxygen intake varied from 3.5 to 4.5 ml/min/kg body weight, and no metabolic elevation was observed at an ambient temperature of 25° C and higher. The oxygen intake at 20° C was markedly increased. Our data show that the critical temperature for a naked man lying stationary is about 25° C. The skin temperature is a function of the environmental temperature. After 2—3 hours' initial fall, the rectal temperatures reached a steady state in two of the subjects, at all ambient temperatures, but dropped consistently in one subject at the lowest air temperatures, showing that this subject met a cold situation by drawing on his stored body heat in addition to increasing the body insulation. This mechanism has been seen in many cold habituated people, and our findings indicate that the difference between cold-exposed and non-acclimatized people is quantitative rather than qualitative.

The critical temperature of the naked man, i. e. the lowest ambient temperature at which the normal thermal steady state can be maintained at the basal heat production, has been studied by ERIKSON *et al.* (1956) and SCHOLANDER *et al.* (1957). They determined the critical temperature by having the naked subjects bicycling at low temperatures with rates just sufficient to maintain a constant rectal temperature. From the figures for oxygen intake at those work loads and the basal metabolic rate, the critical temperature was determined by interpolation and found to be about 27° C.

In addition to the unavoidable inaccuracy introduced by the interpolation, this method for determination of the critical temperature is open to criticism because the bicycling produces air-movements around the body which increases the cooling power of the environment. If the subjects are stationary, one would expect to find a larger zone at which the body can maintain the normal thermal steady state at the basal metabolic rate.

This hypothesis was tested by having three young men sleeping and resting during nights at various ambient temperatures, and measuring their metabolic and thermal responses during these conditions.

Methods

The studies were performed by having the subjects lying naked except for cotton shorts on a bed with no under insulation, in a room temperature regulated to ± 0.5° C. Air movement did not exceed 40 feet per min.

The oxygen consumption was measured continuously during the whole night by using an open circuit system. The subject rested his head in a plastic hood which was sealed air-tight to the neck by means of a rubber sleeve and ventilated so that the CO_2-concentration was always kept below 1.0 %. Fresh air was drawn through the hood by means of a Franz Müllers gasometer on which was mounted an electric motor which rotated the slides. The meter was thus converted to an air pump. The gasometer-reading was recalibrated and frequently checked by passing the total air into a large calibrated respirometer tank, a necessary procedure as the original calibration was about 20 % too low. The gasometer has a small membrane pump which takes out a small portion (0.3 %) of the total air passing through the meter. The accuracy of this aliquot sampling was managed by collecting the total gas into a spirometer, and comparing the gas content of the samples given from the membrane pump.

This test showed that the CO_2 and O_2 percentage in the sampling air did not differ more than 0.05 % from the spirometer value.

Eight periods, each of 60 min of continuous sampling, were used, and the O_2 and CO_2 contents of these gas samples were measured by using Scholanders 1/2 ml gas analyzer.

The skin and rectal temperatures were measured by using copper-constantan thermocouples and a single range potentiometer. The thermocouples were calibrated against a mercury thermometer, and the accuracy of the measurements was found to be ± 0.2° C.

Eight different skin temperatures were taken, the same as used by HAMMEL *et al.* (1959). Average skin temperature was calculated by using the following weighing meters:

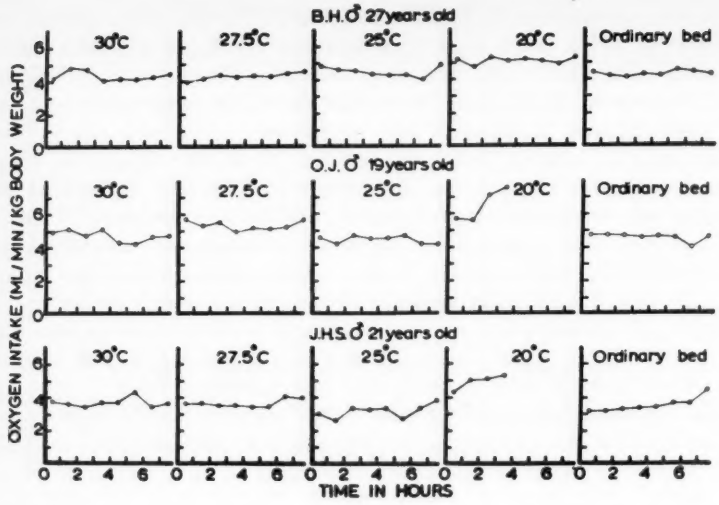

Fig. 1. Oxygen intake in naked young men when sleeping or resting, at various ambient temperatures.

dorsum of foot	0.07
lateral side of lower leg	0.13
lateral side of thigh	0.19
pectoral	0.18
scapula	0.17
lateral side of upper arm	0.14
dorsum of hand	0.05
forehead	0.07

The thermocouples were attached to the skin by means of a single layer of adhesive tape, 2 × 2 cm (Sleek).

The rectal temperature was measured by covering the thermocouple with a plastic tube and inserting the measuring cell about 10 cm inside the anus.

The diet of the subjects was controlled the day preceding the experiment in as much as they were told to consume protein-poor food for breakfast and lunch. At 3 p. m. they were given a light meal of about 1000 kcal, and containing about 60 g of protein. At 7 p. m. they were given a light meal of about 750 kcal containing about 12 g of protein.

The experimental period started at 9.30 p. m. by the attaching of the thermocouples and placing the subjects on the experimental bed. The metabolic period started each evening at 10 p. m. and lasted for eight hours.

Each subject was studied during four nights at various ambient temperatures. One additional night was spent in an ordinary bed, furnished with usual bedclothes, and the room temperature was kept at a comfortable level — mostly at 20° C.

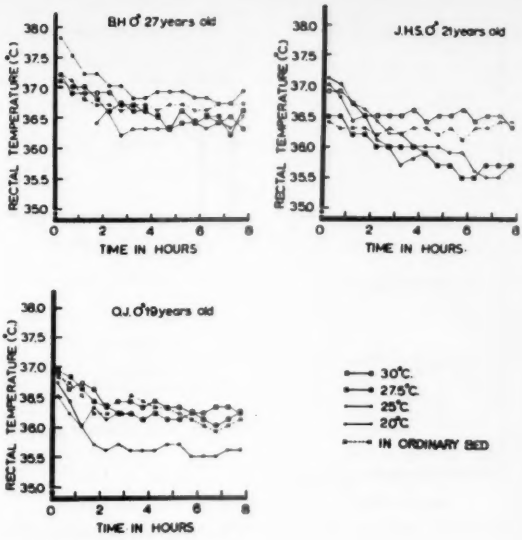

Fig. 2. Rectal temperatures.

Results

The figures for oxygen consumption are given as ml/kg body weight/minute. The results of all the experiments are shown in Fig. 1.

When the subjects were lying in ordinary beds at a comfortable temperature level they slept well and their oxygen intake varied little during the night, but there existed a considerable interindividual variation. Subject J. H. S. had a mean oxygen intake of 3.5 ml/min/kg body weight (range: 3.2—4.5). The corresponding values for subjects O. J. and B. H. respectively were: mean 4.6 ml/min/kg body weight, and 4.3 ml/min/kg body weight (range: 4.0—4.8 ml/min/kg body weight and 4.1—4.6 ml/min/kg body weight).

All subjects were uncomfortable at 30° C because it was too hot. At 25° C they all felt a little chilly, but the cold stress was insufficient to raise the metabolic rate measurably. At 20° everybody suffered from the cold, and two of the three subjects could not stand the cold stress for more than four hours. During this period the metabolic rate was increased above the normal level.

The rectal temperatures are shown in Fig. 2. An initial fall was seen during the first 2—3 hours of sleep or rest, after which the temperature levelled off with exceptions for subject J. H. S. Though there was a tendency for lower end temperatures with lower environmental temperatures, exceptions were so frequently seen that no safe conclusions on this problem can be drawn from the data.

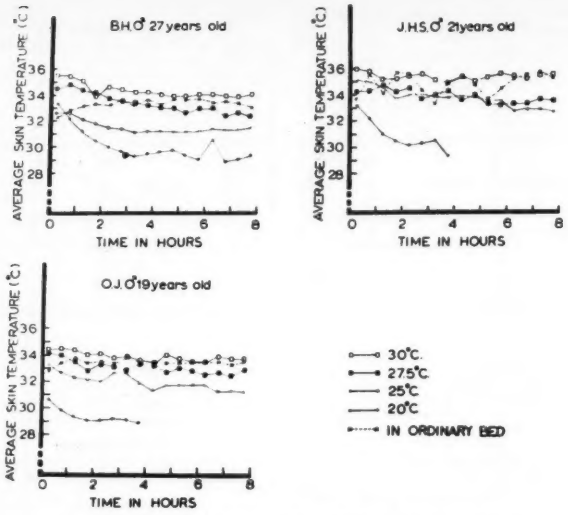

Fig. 3. "Average skin temperature" based on eight different skin-surface temperatures of the body.

The "average skin temperature" is given in Fig. 3, while Fig. 4 shows the skin temperature on the feet. It is quite obvious that lower environmental temperatures give lower skin temperature.

Discussion

Although the small number of subjects investigated limits the generalizations which can be drawn from our data, it is quite obvious that the metabolically neutral zone extends below 27°C, the critical temperature for the naked man found in bicycle experiments by earlier investigators. But also for a naked man lying quiet the critical temperature is quite high, close to 25° C, which supports further evidence for the statement that man thermally behaves as a tropical mammal.

The thermal comfort zone for the naked resting man is surprisingly small. Our subject felt uncomfortably warm when sleeping and resting at an ambient temperature of 30° C and chilly at 25° C in a room where air movements did not exceed 40 feet per min, and relative humidity was below 50 %. At 20° C the cold stress was felt so severely that two of our subjects gave up after four hours exposure.

In earlier studies on human adaptation to cold, altogether eight unacclimatized naked men were exposed to a similar cold stress. They all endured the eight hours cold exposure, but were considerably bothered by the cold, were unable to sleep and their metabolic rate was increased, on average 20—

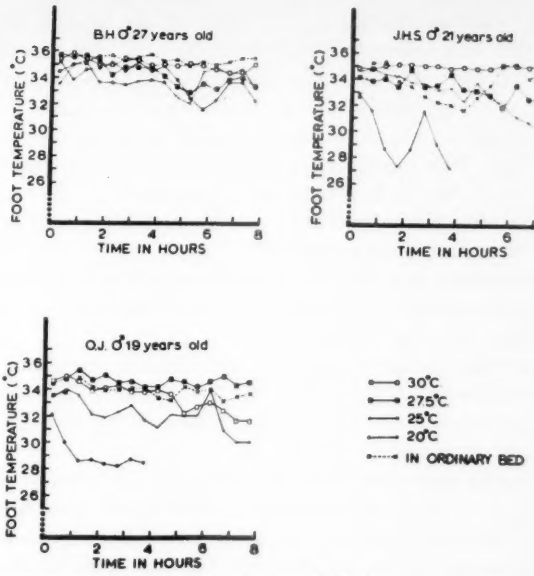

Fig. 4. Skin-surface temperatures on the dorsum of the feet.

60 % above the basal level; they may therefore have the same comfort zone as our subjects. SCHOLANDER *et al.* (1958) exposed eight young subjects who were acclimatized to cold weather, to the same 20° C cold room for eight hours and found that these subjects were considerably less disturbed by the cold. Studies undertaken of cold-exposed people, like the aborigines in the Australian inland (5), the nomadic Lapps in the north of the Scandinavian peninsula (ANDERSEN *et al.* 1960), and the Arctic Indians in Alaska and Northern Canada (IRVING *et al.* 1960) also indicate that habituation to life in the cold may extend the comfort zone some few degrees centigrade below that seen in unacclimatized subjects, although this problem has never been systematically investigated.

When man is exposed to falling ambient temperatures, he usually meets this situation first by increasing the body insulation, and later by increasing the metabolic rate in order to keep the body temperature constant. This mechanism is clearly demonstrated in two of our experimental subjects (O. J. and B. H.). After 2—3 hours' initial fall in the rectal temperature, this stays fairly constant throughout the experiments at all environmental temperatures, while the skin surface temperature decreases with lower ambient temperatures.

For subject J. H. S. the mechanism seems to be different. He also increases body insulation by lowering skin surface temperature at lower ambient temperatures, but the rectal temperature for this subject does not level off after

the initial drop, but falls steadily in the course of the experimental period. This indicates that this subject meets a moderate cold exposure by drawing on the stored body heat in addition to increasing the body insulation before the cold stress is so severe that it starts a metabolic compensation. This subject had the lowest metabolic rate of all the three subjects, but his thermal comfort zone was the same. This physiological mechanism has been found in many people habituated to life in the cold, most pronounced in the aborigines in the Australian inland (SCHOLANDER *et al.* 1958), and is the way these people extend their comfort-zone downwards.

That this mechanism also appears among unacclimatized subjects shows that the difference between cold-adapted and unacclimatized subjects is quantitative rather than qualitative.

The oxygen intake during the night at comfortable temperature levels is fairly constant, and does not vary much from night to night. The interindividual variability is, however, considerable, ranging from an average of 3.5 ml/min/kg body weight to 4.5 ml/min/kg body weight.

The oxygen intake from hour to hour during the night did not follow any consistent pattern — and the drop during the early hours of the night and the rise towards the morning described by other investigators (ANDERSEN 1960) was not present in most of the experiments. KREIDER *et al.* (1958), however, also found a variable pattern of oxygen consumption in four of nine men. The mean values of oxygen intake during the night at comfortable ambient temperatures are in accordance with the values found in other investigations (KREIDER *et al.* 1958, IRVING *et al.* 1960) though in the upper part of the range.

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